DEVELOPMENT OF AN ON-SITE SAMPLING AND EXTRACTION APPROACHES FOR ORGANOCHLORINE PESTICIDES IN SEAWATER AND SEA FOODS

BY

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This thesis is dedicated

To:

My beloved mother for her prayers and my brothers and sisters

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TABLE OF CONTENTS

LIST OF FIGURES

Table Page

LIST OF TABLES

Table page

THESIS ABSTRACT

NAME : **OMAR MOHAMED HUSSSEIN**

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Organochlorines pesticides (OCPs) is a class of pesticides that have been prohibited worldwide since the beginning of 1980s due to their toxicity, stability, high liposolubility, long biological half-life, and consequently a high degree of bioaccumulation in food chain. The pollutants can be transferred to animals from either the surrounding environment or from diets. Also, the lipid content of animal influences the bioaccumulation process, with that in mind the thesis conducted a study of OCPs on biota samples collected from the supermarkets and fishermen on the eastern province of Kingdom Saudi Arabia. A novel on-site sample preparation approach for the OCPs using micro-solid phase microextraction with a battery operated continuous flow device has been developed, this handheld battery-operated flow pump was used to provide agitation of the sample solution at the sampling site to facilitate extraction. The proposed system was developed to meet the demand for an effective, efficient, and affordable on-site large volume sampling tool for monitoring of pollutants in the sea water.

ABSTRACT (ARABIC)

ملخص الرسالة

المبيدات الكلورية العضوية (OCPs) هي فئة من المبيدات التي تم حظرها في جميع أنحاء العالم منذ عام 1980 و ذلك بسبب سميتها، وعدم استقرارها الكيمائي و قابليتها العالية في الذوبان في المحيط الدهني. إضافة الى طول نصف-عمرها البيولوجي. وبالتالي فإن هذه المبيدات تتراكم بنسبة عالية في السلسلة الغذائية للكائنات الحية. و بالامكان ان تنتقل مكونات هذه المبيدات إلى الحيوانات عن طريق البيئة المحيطة أو من الوجبات الغذائية. كذلك، و بما ان محتوى الدهون الحيواني يؤثر على عملية التراكم، قد أخذنا ذلك في الاعتبار و أجرينا دراسة على المبيدات الكلورية العضوية المستخلصة من عينات لانسجة الكائنات الحية و التي تم جمعها من محلات السوبر ماركت وصيادي الأسماك في المنطقة الشرقية من المملكة العربية السعودية. وفي هذه الرسالة تم تطوير الية جديدة لاعداد العينات في الموقع(خارج المعمل) لهذه المبيدات و ذلك باستخدام جهاز مزود ببطارية كهربائية و الذي يتمتع بخاصية التدفق المستمر حيث صمم للاستخراج الصلب جزئيا للاجسام الصغيرة. ويستخدم هذه الجهاز الكهربائي المحمول (المضخة) لتهيج و اثارة محلول العينة في موقع أخذ العينات و ذلك لتسهيل عملية الاستخراج. وقد تم تطوير الجهاز المقترح لتلبية الحاجة الى الحصول على عينات كثيرة في مساحة شاسعة وبكفاءة عالية و بأسعار معقولة. هذا من شأنه ان يسهل عملية رصد الملوثات الكيمائية في مياه البحر.

NOMENCLATURE

C_2	-	Ethyl
C_8	-	Octyl
C_{18}	-	Octadecyl
ECD	-	Electron Capture Detector
DDT	-	Dichlorodiphenyltrichloroethane
DLLME	-	Dispersive Liquid – Liquid Micro-Extraction
DNA	-	Deoxyribonucleic acid
GC-ECD	-	Gas Chromatography - Electron Capture Detector
GC-MS	-	Gas Chromatography Mass Spectrometer
HCBs	-	Hexachlorobenzenes
HCHs	-	Hexachlorocyclohexanes
LLE	-	Liquid- Liquid Extraction
LOD	-	Limit of Detection
LOQ	-	Limits of Quantification
LRAT	-	Long Range Atmospheric Transport
MRL	-	Maximum Residue Limit
mg L^{-1}	-	Millilgram per litre (10^{-6})
ng g^{-1}	-	Nanogram per gram(10^{-9})
ng L^{-1}	-	Nanogram per litre (10^{-12})
OCPS	-	Organochlorine pesticides
PCBs	-	Polychlorinated biphenyls

POPs	-	Persistent Organic Pollutants
ppb	-	Parts per billion (10^{-9})
ppm	-	Parts per million (10^{-6})
SPE	-	Solid phase extraction
USEPA	-	United states environmental protection agency
µg /g	-	Microgram per gram (10^{-6})
µg /kg	-	Microgram per kilogram (10^{-9})
µg L^{-1}	-	Microgram per litre(10^{-9})
µL	-	Micro liter
µ-SPE	-	Micro solid phase extraction
α-BHC	-	α-Benzenehexachloride

CHAPTER 1: INTRODUCTION

INTRODUCTION

1.1 PESTICIDES

1.1.1 Historical aspect of Persistent Organic Pollutants(POPs)

The industrialization of the world has brought us an overwhelming variety of new materials, technologies and products, creating high living standards that certainly no one could imagine a hundred years ago. The process has released and continues to release an enormous diversity of chemicals into the global environment. Currently, approximately 80,000 chemicals (although the exact figure is unknown) are produced, marketed, used, and disposed of worldwide. Each year, hundreds of new chemicals are added to this ever-growing list [1].

Many of these chemicals were praised inventions in their time but have later proved to be disastrous for the environment. For example the creation of DDT (1, 1, 1-trichloro-2, 2-bis (4-chlorophenyl) ethane) by Paul Muller, was awarded the Nobel Prize in Medicine in 1948 due to its great impact on insect-borne diseases [2]. polychlorinated biphenyls (PCBs) are very good electrical insulators as well as highly non-flammable. Due to their dielectric properties, they found extensive use in electronic industry, where they were utilized as insulating additives in circuit boards, capacitors, transformers, and other electrical components [3]. Commercial production of PCBs began in 1929 [4]. PCBs were first recognized as an environmental threat by the Swedish scientist Soren Jensen in 1966, when he found the substances in fish, birds and humans [4].

These compounds of the industrialization epoch are nowadays called pollutants and can be found virtually anywhere all over the world. They are collectively known as Persistent Organic Pollutants (POPs).

The World Health Organisation has classified active ingredients of pesticides according to their acute toxicity. Those which are considered to be most hazardous are placed in the Class, "Extremely Hazardous"; [5]

Pesticides can be classified by target organism, chemical structure, and physical properties. Pesticides can also be classed as inorganic, synthetic, or biopesticides, although the distinction can sometimes hard. Subclasses of pesticides include: herbicides, insecticides, fungicides, rodenticides, pediculicides, and biocides. Many pesticides can be grouped into chemical families. Prominent insecticide families include organochlorines, organophosphates, and carbamates. [5].

Organochlorine pesticides have a long history of widespread use around the world. These compounds are typically very persistent in the environment, and are known for accumulating in sediments, plants and animals. Organochlorines have a wide range of both acute and chronic health effects, including cancer, neurological damage, and birth defects. Many organochlorines are also suspected endocrine disruptors. [4].

Organochlorine pesticides (OCPs) belong to an important class of persistent organic pollutants (POPs) which are endocrine disrupting. They were widely used in agriculture between the 1950s and 1970s. There are several reasons why OCPs have been gathering much international concern. They are very persistent and remain in the environment, despite the fact that many of

them have been banned for decades. Being hydrophobic, they prefer to exit aqueous medium to accumulate in organic matter such as sediment, hence encouraging bioaccumulation through bio-concentration and ingestion. They are also lipophilic; easily concentrate in fats of animals, which leads to biomagnifications. In addition, their endocrine disrupting nature, which has adverse effects on the reproductive system and/or the immune system, is biologically active even when they are present at concentrations as low as 10^{-9} M. Therefore, it is essential to monitor the contaminant level of these OCPs in the environment. [4, 6].

It appears that the more volatile a chemical, the greater tendency it has to remain airborne and the faster and farther it travels on air currents towards remote Polar Regions. Conversely, chemicals of low volatility are unable to attain high atmospheric levels and are thus deposited close to where they are initially released. Therefore, POPs of higher volatility like α- and γ- HCH may migrate faster towards the poles than those of lower volatility like DDT which tend to remain closer to their source [7, 8].

Observations suggest that certain POPs such as HCBs and HCHs, preferentially deposit in polar latitudes, while DDT and others primarily deposit at lower latitudes [7, 8]. For example, a worldwide study of persistent organochlorines in tree bark found that the relatively volatile compounds HCB were distributed according to latitude, demonstrating a global distillation effect. Conversely, less volatile compounds such as endosulfan were not as effectively distilled and tended to remain in the region of use [7, 8].

Being one of the broad-spectrum pesticides, use of OCPs was widely against vegetal pests and vector borne diseases, thereby highly contaminating the water and soil environments [9].

Studies involving of OCPs for the determination in environmental matrices normally deal with samples with low analyte concentrations containing a high number of interfering compounds. Thus, simple and highly sensitive analytical techniques are required to detect and quantify pollutants in water at trace levels [10].

1.1.2 Ecological effects of pesticides

Pesticides are included in a broad range of organic micro pollutants that have ecological impacts. Different categories of pesticides have different types of effects on living organisms, therefore generalization is difficult. Although terrestrial impacts by pesticides do occur, the principal pathway that causes ecological impacts is that of water contaminated by pesticide runoff. The two principal mechanisms are bioconcentration and biomagnification. [11]

Bioconcentration: This is the movement of a chemical from the surrounding medium into an organism. The primary "sink" for some pesticides is fatty tissue ("lipids"). Some pesticides, such as DDT, are "lipophilic", meaning that they are soluble in, and accumulate in, fatty tissue such as edible fish tissue and human fatty tissue. Other pesticides such as glyphosate are metabolized and excreted. [9]

Biomagnification: This term describes the increasing concentration of a chemical as food energy is transformed within the food chain. As smaller organisms are eaten by larger organisms, the concentration of pesticides and other chemicals are increasingly magnified in tissue and other organs. Very high concentrations can be observed in top predators, including man. [11]

The ecological effects of pesticides (and other organic contaminants) are varied and are often inter-related. Effects at the organism or ecological level are usually considered to be an early warning indicator of potential human health impacts. The major types of effects are listed below and will vary depending on the organism under investigation and the type of pesticide. Different pesticides have markedly different effects on aquatic life which makes generalization very difficult[10]. The important point is that many of these effects are chronic (not lethal), are often not noticed by casual observers, yet have consequences for the entire food chain. [6]

- Death of the organism. [8]
- Cancers, tumours and lesions on fish and animals. [9]
- Reproductive inhibition or failure. [2]
- Suppression of immune system. [6]
- Disruption of endocrine (hormonal) system. [8]
- Cellular and DNA damage. [1]
- Teratogenic effects (physical deformities such as hooked beaks on birds). [8]
- Poor fish health marked by low red to white blood cell ratio, excessive slime on fish scales and gills, etc. [6]
- Intergenerational effects (effects are not apparent until subsequent generations of the organism) [9].
- Other physiological effects such as egg shell thinning [11].

With that in mind we developed a novel on-site sample preparation approach using micro-solid phase extraction (μ- SPE) for the OCPs determination from water. In the second part of thesis embarks on the determination of OCPs in biota samples from the coastal areas and supermarkets of the eastern province of Saudi Arabia.

1.2 OBJECTIVES

The main objectives of the thesis are;-

- Development of an on-site extraction method for large volume seawater samples.
- Application of developed on-site method for the determination of OCPs in seawater samples.
- Determine OCPs in various sea foods from local market and supermarkets in the eastern province of the Saudi Arabia.

1.3 PROBLEM STATEMENT

The Stockholm Convention on Persistent Organic Pollutants(POPs) United Nations treaty, has established global bans on several organochlorine pesticides including DDT, hexachlorobenzene, pentachlorobenzene, chlordane, dieldrin, endrin, heptachlor, mirex, toxaphene, hexachlorocyclohexane (alpha-HCH, beta-HCH, and gamma-HCH (lindane), and chlordecone.Saudi Arabia is a signatory to the Stockholm convention however there is no regular POPs data or reported monitoring data in the literature. [1].

The process of sampling while representing effectively the area of analysis is difficult especially in the monitoring of environmental pollutants in water. The proper sampling leads to accurate analysis in the final run and thereby leading to appropriate measures to curb the pollution level. Pollution may be occurring on the coastline and lack of proper and well elaborated sampling may lead pollution affecting environment.

The monitoring of OCPs is an important aspect of safeguarding the life of both marine and terrestrial life of living things on it. It is with this in mind that we are proposing a sampling and extraction approach using a novel idea and analysis of sea foods in eastern province of the kingdom of Saudi Arabia.

1.4 IMPORTANCE OF STUDY

Although the use of pesticides has resulted in increased crop production and other benefits, it has raised concerns about potential adverse effects on the environment and human health. The greatest potential for unintended adverse effects of pesticides is through contamination of the hydrologic system, which supports aquatic life and related food chains and is used for recreation, drinking water, irrigation, and many other purposes. Water is one of the primary pathways by which pesticides are transported from their application areas to other parts of the environment. [9,10].

Aquatic biota also is important in the food web of terrestrial organisms, with some aquatic biota, such as fish, being consumed by people and wildlife. Analyzing contaminants in aquatic biota provides an efficient way to test whether hydrophobic contaminants are present in the stream. Many hydrophobic chemicals also are resistant to degradation, so they persist for a long time in the environment. [10].

Persistent hydrophobic contaminants in a stream or ocean water may accumulate in aquatic biota, even when concentrations in the water are too low to be detected using conventional sampling and analytical methods. [7].

Due to trace level concentration in the environment, conventional extraction techniques are not suitable choice analytes will be lost due to multi step procedures. Therefore we propose to develop simple methods for OCPs in sea water and sea food samples.

Chapter 2: Literature Review

2. Literature Review

Introduced in the 1940s, organochlorine pesticides (OCPs) were widely used in agriculture and pest control until research and public concern regarding the hazards of their use led to government restrictions and bans. Despite restrictions and bans on the use of many organochlorine pesticides in the 1970s and 1980s, they continue to persist in the environment today. Organochlorine pesticides are hydrocarbon compounds containing multiple chlorine substitutions. There are four main types of Organochlorine pesticides; dichlorodiphenylethanes; cyclodienes; chlorinated benzenes; and cyclohexanes. All share a similar pair of carbon rings, one ring being heavily chlorinated. [12].

2.1 Properties and sources of (OCPs)

Persistent organic pollutants (POPs) are those chemicals that are not materially broken down over a reasonable period of time, usually measured in decades or more. Throughout history, various types of pests, such as insects, weeds, bacteria, rodents, and other biological organisms, have bothered humans or threatened human health. People have been using pesticides for thousands of years to try to control these pests. The Sumerians used sulfur to control insects and mites 5,000 years ago. [13]. The Chinese used mercury and arsenic compounds to control body lice and other pests. The Greeks and Romans used oil, ash, sulfur, and other materials to protect themselves, their livestock, and their crops from various pests. And people in various cultures have used smoke, salt, spices, and insect-repelling plants to preserve food and keep pests away. Approximately 90 percent of all pesticides used worldwide are used in agriculture, food storage,

or shipping. Because of a growing world population, there is pressure to increase and preserve the food supply by using pesticides and other agricultural chemicals. [13]

The organochlorine pesticides are generally stable in the environment and undergo limited decomposition or degradation. Organochlorine pesticides are not particularly volatile, but because they tend to persist in the environment, they can cycle among air, water, soil, vegetation, and animals. Organochlorine pesticides can travel long distances via wind and deposit on soil and water, so they can be found hundreds or thousands of miles from their point of use. They can also be transported on foods and other products treated with them. Because these organochlorine pesticides are fairly non-polar molecules, they tend to dissolve readily in hydrocarbon-like environments, such as the fatty material in living matter. [14].

They are only slightly soluble in water. Although organochlorine pesticides can evaporate into the air, they adhere strongly to soils or sediments, where their concentrations can build up, often exceeding those of surrounding water by orders of magnitude. Organochlorine pesticides in water and sediments tend to bioaccumulate in living tissues, particularly in fish and other aquatic organisms. They also bioaccumulate in plants, birds, terrestrial animals, agricultural livestock, and domestic animals, where their concentrations increase by orders of magnitude as they rise through the food web, particularly as they reach higher organisms. At low concentrations, organochlorine pesticides exhibit relatively low acute toxicity to humans; however, they may mimic human hormones like estrogen, or have other properties that cause long term health effects. At higher concentrations, organochlorine pesticides can be very harmful, causing a range of problems including mood change, headache, nausea, vomiting, dizziness, convulsions, muscle tremors, liver damage, and death [15]. As a result of observed effects on animals and plants in

the environment, and potential harmful effects to humans, many uses of organochlorine pesticides have been banned [14].

2.2 Distribution of Organochlorine Pesticides

One peculiarity of the global persistent organic pollutants (POPs) distribution is their accumulation in the environmental compartments situated at higher latitude, resulting in an enrichment of the concentrations of some POPs in polar ecosystems to levels that sometimes exceed human consumption guidelines. [15].

Organochlorine pesticides (OCPs) are typical (POPs). They are of worldwide concern owing to their persistence, bioaccumulation, and potential negative impacts on humans and wildlife. OCPs are subject to global redistribution through the environment via long range atmospheric transport (LRAT), or by ocean currents and animal migration [16].

Large water bodies such as oceans and seas play an important role in the global biogeochemistry of POPs [17], either acting as a sink [18m] or as a source for POPs in the environment. Diffusive exchange of POPs across the air−water interface may alter direction as a consequence of global/regional reduction of POP sources [19]. The evaluation of air−sea equilibrium status of POPs in different regions is therefore critical to understanding their global source/sink contributions.

2.3 Organochlorine Pesticides In The Physical Environment

2.3.1 Organochlorine Pesticides in Soil

Sources of pesticide in soil are mainly the following aspects: using for preventing pest; the input of irrigation water and the deposition of atmospheric particles. In recent years, due to rapid growth of food demand, pesticide applications in most cases are excessive. Besides, solid wastes are piled up and dumped to the soil surface continually, thus, hazardous wastewater is continued to infiltrate into the soil, more and more hazardous gases and particulates landed into the soil with rain. When the content of harmful substances in soil exceed the soil's self purification ability, the composition, structure and function of soil will be changed, microbial activity will be inhibited, and harmful substances or its decomposition products will be accumulated in soil gradually, finally absorbed by human body, when the extent great enough to threaten human health, the soil pollution is formed. [20].

Monitoring of OCPs was carried out to identify and quantify the contribution of point and nonpoint sources to the total OCP flux in a southeastern region of Argentina. Results show that, although most of these pesticides are banned, they are present in these soils and the atmospheric transport and deposition would be the major processes for distributing OCPs from target to natural areas [21].

Concentrations of organochlorine pesticides have been successfully measured in nest soil, complete clutch of eggs, and blood of the common freshwater turtle lived in rice field habitat in the Chao Phraya River Basin, Thailand. The results indicated that although all of these pesticides

had been banned in Thailand for many years, their detectable levels in nest soil and turtle eggs indicate that they can persist in agricultural fields for long period of time. [22].

2.3.2 Organochlorine Pesticides in Water

Monitoring program for pesticides are generally poor in much of the world and especially in developing countries. Key pesticides are included in the monitoring schedule of most western countries, however the cost of analysis and the necessity to sample at critical times of the year (linked to periods of pesticide use) often preclude development of an extensive data set. [23]. Many developing countries have difficulty carrying out organic chemical analysis due to problems of inadequate facilities, impure reagents, and financial constraints. New techniques using immunoassay procedures for presence/absence of specific pesticides may reduce costs and increase reliability. Data on pesticide residues in fish for lipophilic compounds, and determination of exposure and/or impact of fish to lipophobic pesticides through liver and/or bile analysis is mainly restricted to research programmes. Hence, it is often difficult to determine the presence, pathways and fate of the range of pesticides that are now used in large parts of the world [23].

Analysis of water samples from Jeddah Saudi Arabia RO/MSF plants for organic pollutants including pesticides showed trace concentrations were detected in seawater and chlorinated seawater but most of them seem to have been rejected by RO membranes. Little or negligible concentrations of chlorinated hydrocarbons, phenols, pesticides or polynuclear aromatic hydrocarbons were detected in some of the samples tested. [24].

Different methods have been used to determine OCPs in different types of water with methods of extraction and also different techniques of analysis.

Static liquid-phase microextraction, with subsequent analysis by gas chromatography–electron-capture detection, has been applied to extract eight organochlorine pesticides from water. The method was precise, reproducible and linear over a wide range and required only small volumes of organic extractant as well as samples. [24].

Dispersive liquid-liquid microextraction (DLLME) coupled with gas chromatography-electron capture detection (GC-ECD),has been developed for the extraction and determination of fourteen organochlorine pesticides (hexachlorocyclohexanes (HCH, β-HCH and δ-HCH),Lindane (γ-HCH),Aldrin, Dieldrin,Endrin,Heptachlor, Heptachlor epoxide, Chlordane and p,p'-DDT, p,p'-DDD, p,p'-DDE) in river water samples. The method was successfully utilized for the preconcentration and determination of the organochlorine pesticides in the Jajrood River water samples [25].

2.3.3 OCPs in Biota Species

Literature indicates that almost all previous studies monitoring OCPs residues in biota have focused on fish and other organisms with high trophic levels. The present study demonstrates that organisms at mediterean levels also may accumulate high levels of OCPs and consequently can be a health concern to humans and the ecosystem. [26]

The pollutants are transferred to animals from either the surrounding environment or from diets. Also, the lipid content of animal influences the bioaccumulation process. Thus, the determination

of OCPs residues amounts is necessary in animal products. Until now, it is reported that many methods for determination of OCPs residues, such as thin-layer chromatography and high-performance thin-layer chromatography, and Gas Chromatography. Because of liposoluble, low volatile pesticides except HCB, such as OCPs, GC-MS and GC with electro capture detector (ECD) is obviously the preferred approach because of its high sensitivity and selectivity. For GC, different clean-up procedures have been performed to the determination of OCPs, such as liquid-liquid extraction [27] super critical fluid extraction [28] accelerated solvent extraction [29] gel permeability extraction [30] microwave-assisted extraction [31] and solid-phase extraction (SPE). As SPE, florisil, silica, alumina, C-18 materials were used in cleanup step. The SPE requires much lower volume in organic solvent usage is an important advantage. [32]

Study aimed mainly at determining DDTs and PCBs residues in fish and shell fish, and to attempt to identify their major sources and to perform a baseline study on the pollution in the Red Sea of Yemen and Gulf of Aden was conducted [25].The results confirmed that DDTs occurred in almost all fish samples when examined. However, DDTs concentrations were relatively lower than those reported previously in the Arabian Sea [33].

Monitoring of trace toxic substances in the aquatic environment using green mussel (*Perna viridis*) as a biological indicator is commonly used because of its advantages such as the wide geographical distribution, immobile, easy sampling, tolerance of a wide range of salinity and comparatively long life-span. Recent evidence has shown that some chlordane metabolize could be detected in green mussel during 1997–1999. However, the concentrations of residues were lower than the Maximum Residue Limit (MRL) for aquatic animal. [34]

2.4 Toxicity of OCPs

While the public health and economic benefits of synthetic pesticide are indisputable, the findings of widespread environmental contamination by OCPs, reaching global proportions, heralded the end of an era for their extensive use and OCPs have been removed from the market due to their adverse health and environmental effects and their persistence [35].

The health effects of OCPs exposure depend on the specific pesticide, the level of exposure, the timing of exposure and the individual. Different pesticides result in a range of health symptoms.

Highly toxic organochlorines [35]

- ✓ Aldrin
- ✓ Dieldrin
- ✓ Endrin (banned by the US Environmental Protection Agency EPA)
- ✓ Endosulfan

Moderately toxic organochlorines [35]

- ✓ Chlordane
- ✓ DDT (banned by the EPA)
- ✓ Heptachlor
- ✓ Kepone
- ✓ Lindane
- ✓ Mirex
- ✓ Toxaphene

2.5 Metabolism of OCPs

Organohlorine are neuro toxic involved in alteration of ion channels. There are several reports about metabolic disorders, hyperglycemia, and also oxidative stress in acute and chronic exposures to pesticides that are linked with diabetes and other metabolic disorder.

In this respect, there are several in vitro and in vivo but few clinical studies about mechanism underlying these effects. Organochlorine mostly affect lipid metabolism in the adipose tissues and change glucose pathway in other cells. As a shared mechanism, all Organophosphates, Carbamate and Organochlorine induce cellular oxidative stress via affecting mitochondrial function and therefore disrupt neuronal and hormonal status of the body [36].

2.6 Sampling methods of water for OCPs

Sampling could be defined as a process of selecting a portion of material small enough in volume to be transported conveniently and handled in the laboratory, while still accurately representing the part of the environment sampled. The main difficulties in sampling are representativeness and integrity [37].

The frequency of occurrence and the coefficient of variation of a contaminant determine the number of samples required to adequately characterizing exposure pathways, and both are essential in designing sampling plans [36].

Generally there two main sampling methods namely grab and composite sampling. Grab samples are exactly that samples are taken in one go and is the most common form of sampling in

flowing water because it is reliable and easy to do. Mainly is used to provide information about the water at one point in time. Composite sampling involves taking a number of small samples, called sub-samples, over a period of time; these are then combined to reflect the overall condition of a water body, like a lake [36].

The currently used conventional sampling approaches suffer from several limitations:

(1) Spot water samples reflect residue composition only at the moment of sampling and may fail to detect episodic contamination;

(2) quality control and physical difficulties are often encountered when large volumes of water must be collected and extracted for quantifying and assessing trace organic contaminants;

(3) concentrations of truly dissolved contaminants are not accurately measured by most conventional approaches; and,

(4) they are expensive and labor-intensive [38, 39].

In on-site sampling method the sampling takes place on the site and the samples are not transported to the laboratory for extraction and that analysis only is done in the laboratory. On-site methods may be useful for analysis of water. On-site analytical methods are a valuable, cost effective tool to assess the nature and extent of contamination (EPA 1997b). Because costs per sample are lower, more samples can be analyzed. In addition, the availability of near-real-time results permits redesign of the sampling scheme while in the field. On-site analysis also facilitates more effective use of off-site laboratories using more robust analytical methods. On-site methods provide near-real-time feedback, they can be used to focus additional sampling on areas of known contamination, thus possibly saving additional mobilization and sampling efforts. [37].

The accuracy (i.e., the correctness of the concentration value and a combination of both systematic and random error) of on-site measurements may not be as high as in fixed laboratories, but the quicker turnaround and the possibility of analyzing a larger number of samples more than compensates for this potential lack in accuracy [37].

The advantage of the on-site method is that it can minimize errors that are commonly introduced by sample handling, transport, and storage prior to conventional off-site laboratory analysis [37].

Chapter 3: Research Methodology

3.1 Part A: Development of on-site Sampling System for Analysis of OCPs in Seawater

3.1.1 Materials

Flat-sheet Accurel porous polypropylene flat membrane with 0.2 μm pore size was imported from Membrana (Wuppertal, Germany). Various sorbents such as Carbon nanofibers, C_8, and Porapak Type R (divinylbenzene/vinyl pyrrolidinone) were purchased from Alltech (Deerfield, IL, USA) and C_{18} from Phenomenex (Macclesfield, UK). The sampling system comprised of the following: a battery-operated pump (CHINA) which is commercially used as a water dispenser, black rubber O rings, and a water purifying tap designed for home use. The mentioned items are shown in Figure 3.1.1

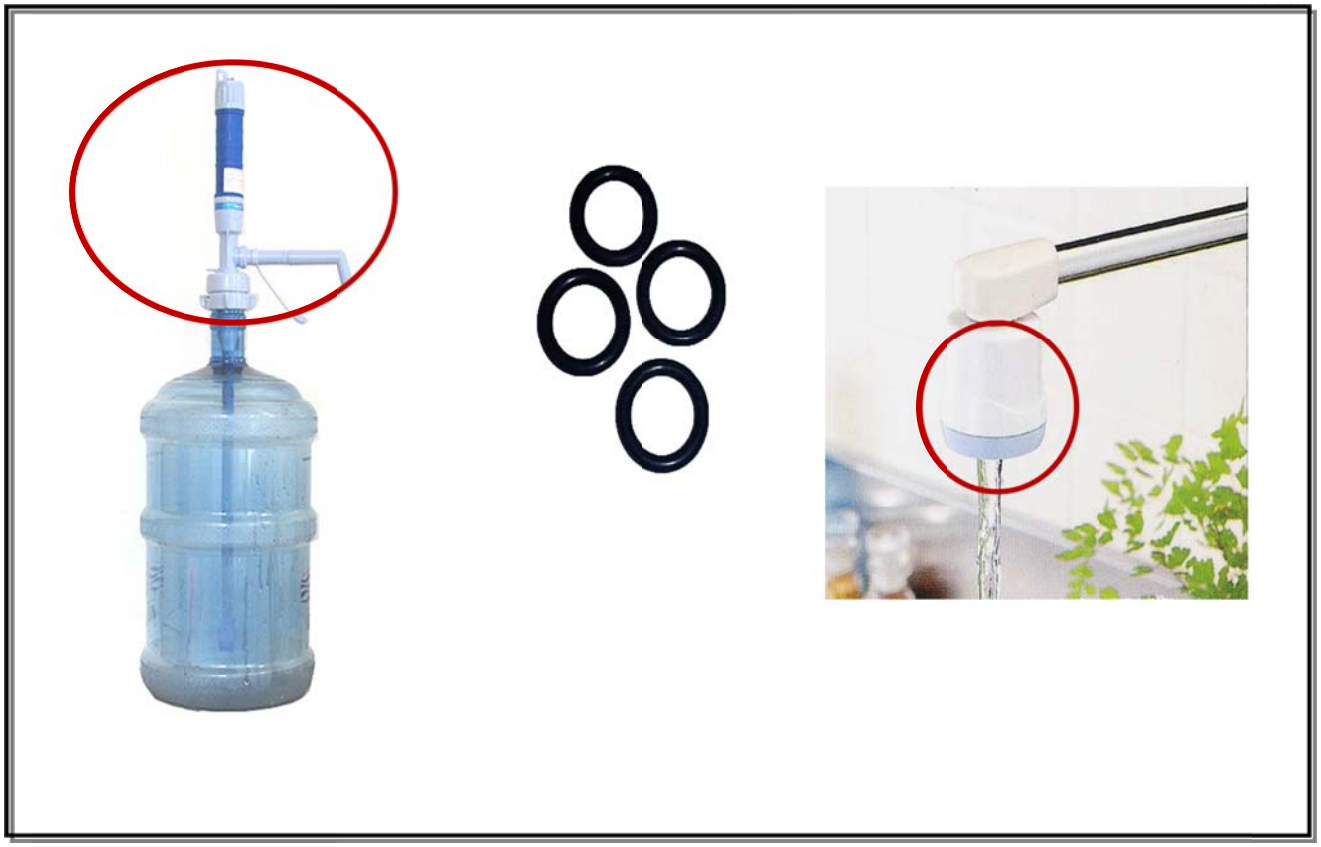


Figure 3.1.1 Materials used in sampling system.

3.1.2 Preparation of μ-SPE Device

The μ-SPE device comprised of a 4.0cm x 4.0cm polypropylene flat-sheet membrane envelope containing a sorbent material. The dimensions of the μ-SPE device were specially measured to fit comfortably into its designated compartment in the sampling system. The preparation process of the membrane envelope was illustrated in Figure 3.1.2. It was made first by cutting out 10.0 cm x 5.0 cm of polypropylene membrane. The membrane was folded over vertically and heat-sealed on two sides. The sorbent material was then introduced via the remaining open end before it was heat-sealed to secure the content. Excess membrane sheet on the sides were trimmed off. After packing, the device was immersed in methanol and cleaned through 10 minutes of ultrasonication. Finally, it was stored in clean methanol until use.

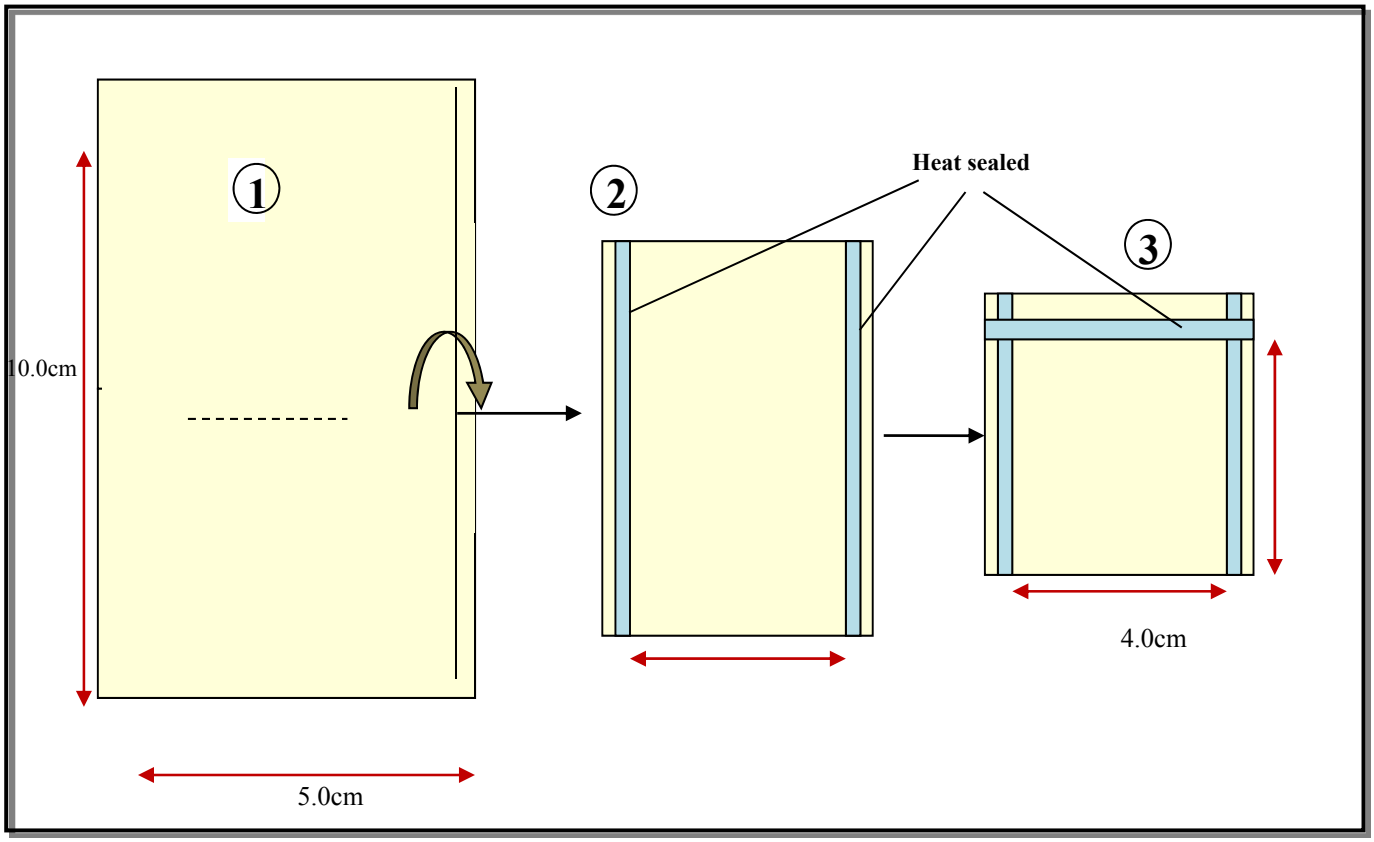


Figure 3.1.2. Preparation of membrane envelope used for μ-SPE procedure.

3.1.3 Sampling System

The materials used for the sampling system were assembled as shown in Figure 3.1.3. The water purifying tap was attached to the battery-operated pump using two rubber O rings. The bottom (blue) part of the water purifying tap served as a lid and could be removed by turning it anti-clockwise. The lid had a hole in the middle to allow water to flow through. A pair of D-size batteries was sufficient to pump about 150 L of water at an approximate speed of 92.8 L hr^{-1}. It is important to avoid introducing moisture into the pump's motor as the pump is not designed to protect its motor from water. Therefore, during sampling, the upper part of the pump (where the motor is) must be held above water level. The part of the pump, which can be immersed in the water, measures about 0.45 m long.

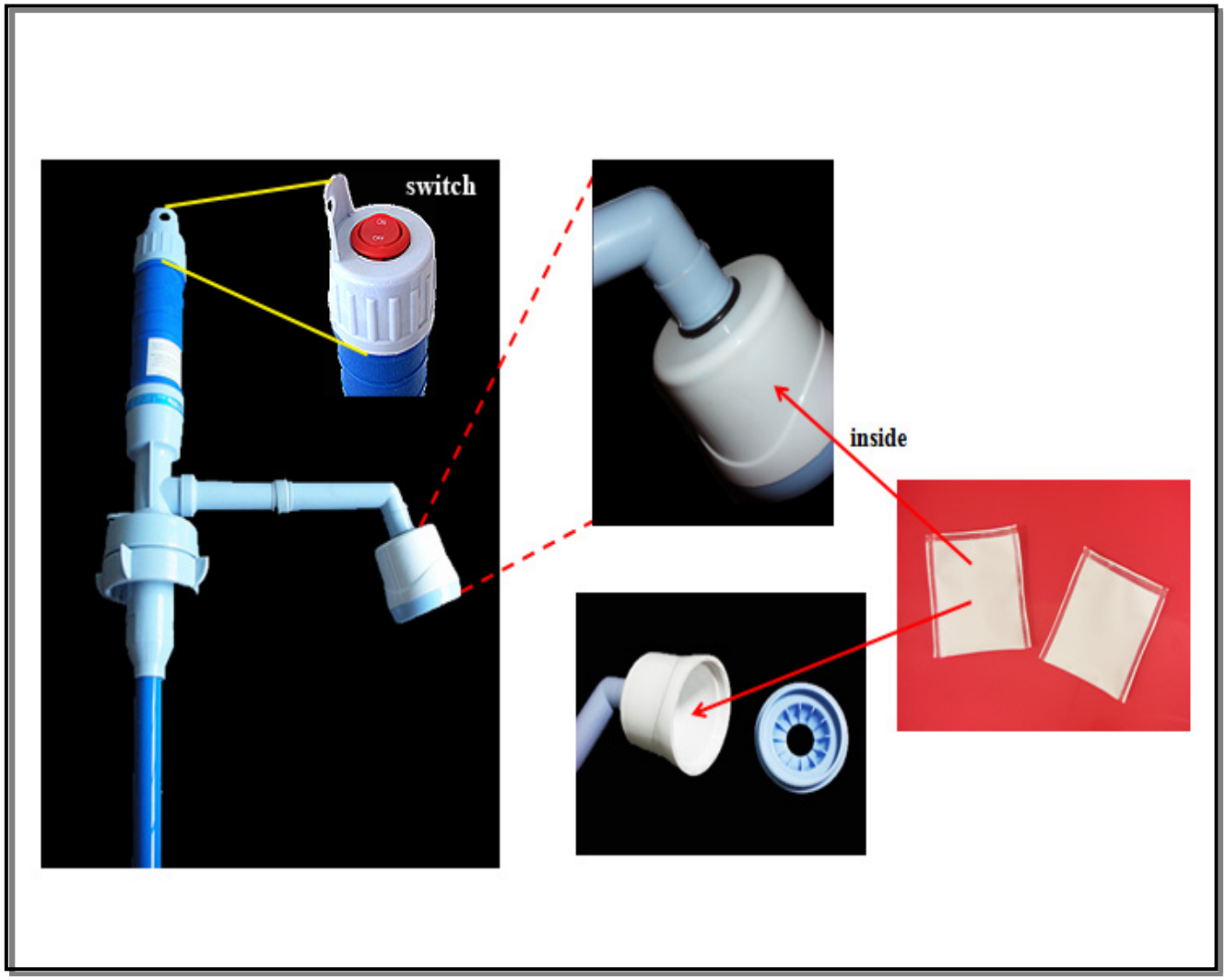
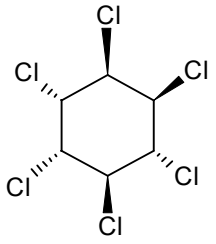


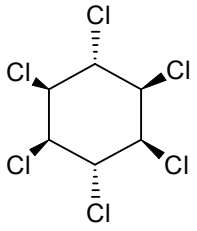
Figure 3.1.3 Details of sampling system.

3.1.4 Target Analyte and Standard Preparation

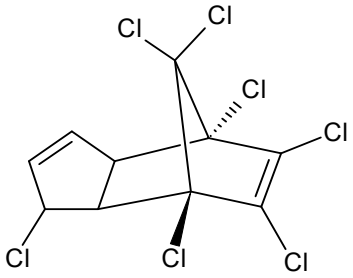
The target OCPs analyte are; α-BHC, Lindane, Heptachlor, Aldrine, Heptachlor epoxide, α-endosulfan, Dieldrin, Endrin, 4,4-DDT,Endosulfan sulfate. Working standard solution of all OCPs target analyte were prepared at 4 different concentration of 0.05, 0.1, 0.5 and 1 ppm.calibration curve was prepared for all target OCPs.The chemical structures of different target analyte are shown in figure 3.1.4



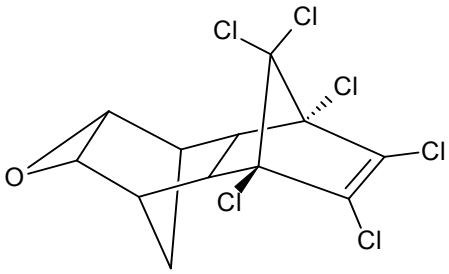
α-BHC



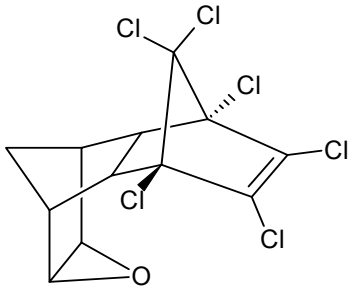
Lindane



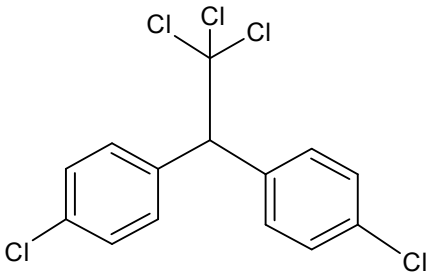
Heptachlor



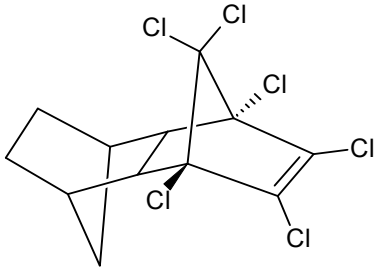
Dieldrin



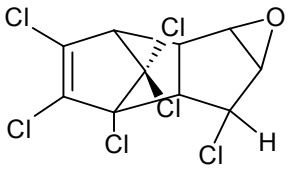
Endrin



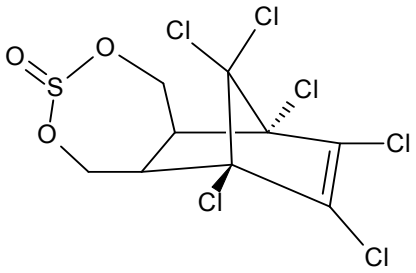
DDT



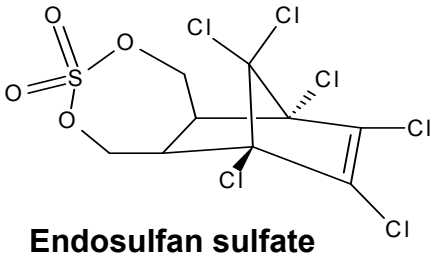
Aldrin



Heptachlor epoxide



α-Endosulfan



Endosulfan sulfate

Fig 3.1.4 chemical structures of target analytes

3.1.5 Study area

The study and sampling area comprised of towns on the coastline of the Arabian Gulf stretching about approximately 300km.The furthest point of sampling was As safaniyah while lowest was khobar as can be seen on figure 3.1.5 below.

Name	Location abbreviation
Safaniya	AG 1
Manifa	AG 2
Khursaniya 1	AG 3
Khursaniya 2	AG 4
Abu ali 1	AG 5
Abu ali 2	AG 6
Abu ali 3	AG 7
Tarut	AG 8
Qatif	AG 9
Dammam 1	AG 10
Dammam 2	AG 11
Dammam 3	AG 12
Dammam 4	AG 13
Khobar	AG 14

Table 3 Sampling location abbreviation

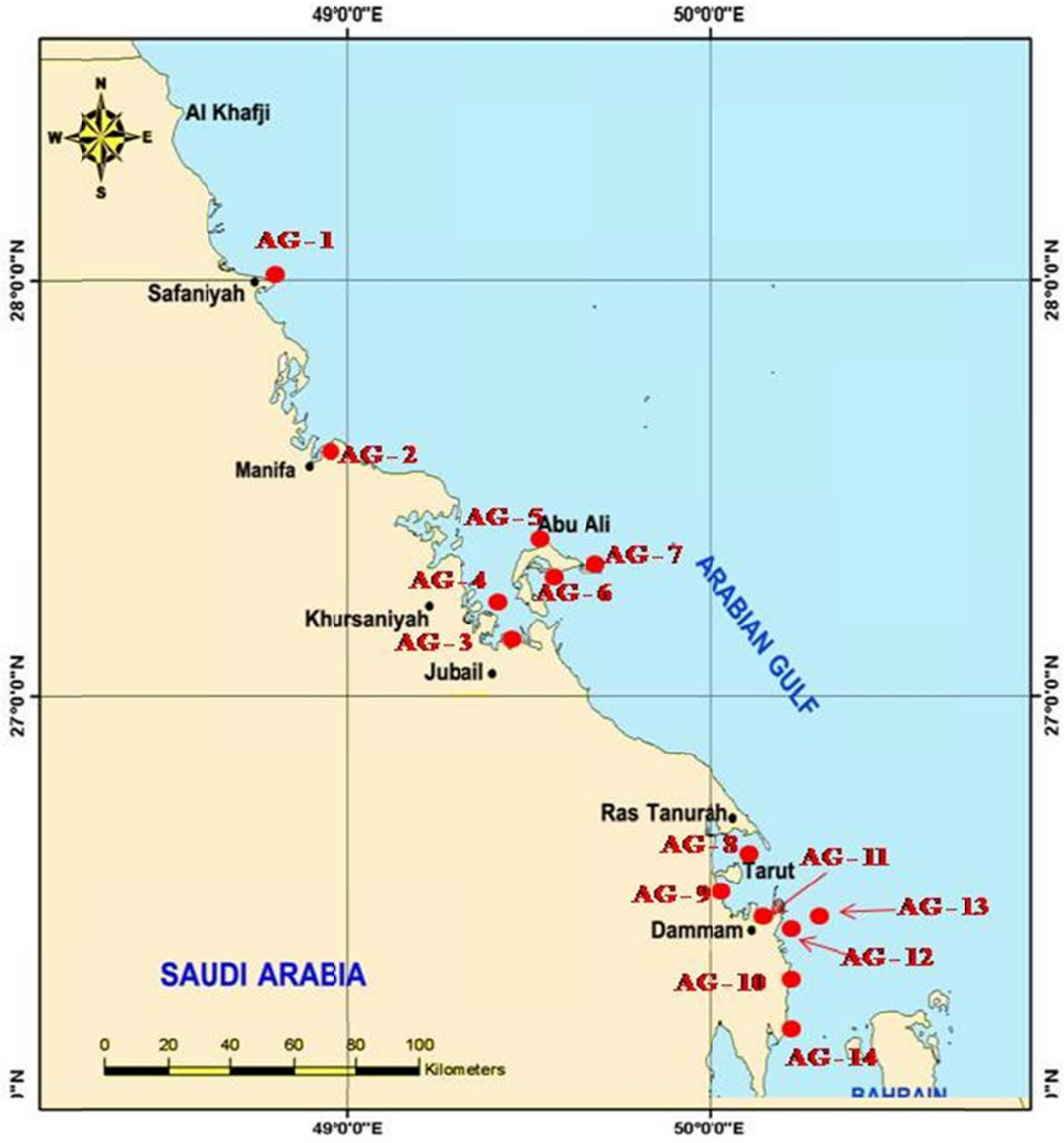


Figure 3.1.5 Sampling on the Saudi Arabian gulf (eastern province)

3.1.6 Method Optimization

Various condition affecting extraction were optimized.10 L of tap water was spiked to contain 50 μg L^{-1} of each target analyte. At any point in time, only 1 parameter was optimized while the others were kept constant. Once a specific parameter was optimized, the value was employed in subsequent optimization.

Using the optimization method different parameters was determined namely;

(a)Type of sorbent: (C_{18} (Octadecyl), C_8 (Octyl), C_2 (Ethyl) and Porapak R sorbent materials were chosen to determine the sorbent material most suitable for use in the μ-SPE device). Peak areas of various types of packing was analyzed and compared.

(b)Desorption Solvent: It was essential to determine a suitable desorption solvent because the partition coefficient of a compound varies in different solvents. Various organic solvents such as n-hexane, toluene, methanol, acetone, and dichloromethane were tested.

(c)Sorbent Amount: The impact of sorbent amount on extraction efficiency was investigated. The amount of sorbent material which could be used for the μ-SPE device is limited by the allowed size of the membrane envelope.

(d)Extraction Time: The amount of analyte extracted depends on the mass transfer of analyte from the sample solution to the solid sorbent material. As mass transfer is dependent on time, the time required to maximize extraction efficiency was evaluated also.

3.1.7 Extraction Process

At an actual sampling site, the μ-SPE device was placed flat on the lid of the water purifying tap before the lid was screwed back onto the tap. The pump was lowered vertically into the sea with its motor and the tap remaining above water level. When the pump was switched on, sea water was drawn up the pump and released through the tap, where it would come into contact with the μ-SPE device.

After extraction, the μ-SPE device was removed using a pair of metal tweezers, dabbed dry with lint-free tissue, and dropped into a glass reagent bottle containing 40 mL of n-hexane. The glass reagent bottle was wrapped with aluminium foil to keep its contents away from sunlight and stored in ice during the journey to the laboratory. Analysis was performed on the same day.

μ-SPE device containing analytes were desorbed via ultrasonication using organic solvent. The analytes were desorbed through ultrasonication for 40 minutes, organic solvent volume was reduced using a gentle stream of nitrogen gas, and the analytes were reconstituted with n-Hexane to 1 mL. Finally 2 μL of the sample was injected into the GC-MS for analysis.

3.1.8 Instrumental Analysis Condition

Analysis was conducted using Agilent (USA) 6890N GC-MS system equipped with Agilent 7683B Series autosampler and a DB-5MS fused silica capillary column (30 m x 0.25 mm I.D., film thickness 0.25 μm, from J & W Scientific, Folsom, CA, USA). The chemical nature of the stationary phase was 95% polymethylsiloxane. Helium was used as the carrier gas with a flow rate of 1.5 mL min^{-1}. The injection port temperature was set at 250^{o}C, and the MS interface

temperature at 270^{o}C. The GC-MS system temperature programme was set as follows: 70^{o}C (hold 1 minute); 15^{o}C/min to 150^{o}C; 2^{o}C/min to 200^{o}C, 15^{o}C/min to 320^{o}C (hold 5 minutes). Samples were injected in splitless mode and the total GC analysis time was 15.33 minutes. A scan range of 80 to 450 m/z was first employed to confirm the retention times of the target compounds, after which selective ion monitoring (SIM) mode was employed to allow higher sensitivity.

3.2 PART B: DETERMINATION OF OCPs IN SEA-FOODS

3.2.1 Sample preparation

In the second part of the thesis, seafood samples were used to determine the concentration of OCPs.Various seafood samples (fish, shrimps, squids, crab and mussels etc) available on the shelves of the main supermarkets in the cities (Dammam, Khobar, Dhahran) were purchased and stored in the freezer (-20 ^{0}c) .Samples were thawed before extraction. During extraction process samples were divided into two portions (liver and the body of the tissue) and extracted for OCPs using USEPA protocol based on sampling protocol [36].

3.2.2 Extraction Process

Amixture of 50 mL of acetone and 50ml deionised was added to the weighed sample in a beaker and homogenized for 5 minutes using an electronic hand blender. The homogenized samples were transferred to a separatory funnel and 20 mL mixture of 1:1(acetone: deionised water) was used to rinse the flask. 20 mL of n-hexane was added. The separatory funnel was shaken for 10 minutes and left to settle. The two layers formed drained into separate conical flasks. The upper layer will be set aside while the lower layer poured back into the funnel. Another 20 mL of n-hexane was poured into the funnel and shaken for 10 minutes. The lower layer was discarded while the upper layer joined the batch previously set aside. Anhydrous sodium sulphate then added to remove excess water.

The mixture was set aside for 15 minutes before being filtered into a round-bottom flask. The filtrate was concentrated to a few mLs using the rotary evaporator at 35^{o}C. Volume of n-Hexane was reduced to 10 Ml and the contents were swirled. The solution was then reduced to a few mLs using the rotary evaporator before being further preconcentrated to about 1 mL using a gentle stream of nitrogen. [40]

The final solution obtained was transferred into a 1 mL GC autosample vial and topup to 1 mL. Finally, 2 μL of the sample was injected into the GC-MS for analysis. [40]

3.2.3 Limits of detection

The limit of detection (LOD) will be defined as 3 times the standard deviation while the limit of quantification (LOQ) will be 10 times the standard deviation. Through triplicates analysis, the LODs and LOQs for the various OCPs will be determined. In addition, analyte with quantity lower than the qualitative limit, given by 5 times the standard deviation, will be taken as not detect. [40]

Chapter 4: RESULTS & DISCUSSIONS

PART I: Development of on-site Sampling System for Analysis of OCPs in Seawater

4.1 System Development

The developed system was able to overcome common problems encountered in large-volume active sampling for water. Its ability to perform on-site sampling also compensated the weaknesses of field sampling. The minute pore size of the membrane used in making the μ-SPE device which served as a filter, preventing particles in the sea water from contaminating and interfering with the extraction. The approximate cost of this system, excluding the μ-SPE device, totalled was less than SAR 150. In addition, the entire sampling system only weighed approximately 500g, including the two D-size batteries. It measured about 27.0 inches at its longest side and 10.0 inches at its widest side. Its light weight and relatively compact design made it portable and easy to store.

4.2 Method Optimization

In carrying out the extraction method optimization, 10 L of tap water was spiked to contain 50 μg L^{-1} of each target analyte. At any point in time, only 1 parameter was optimized while the others were kept constant. Once a specific parameter was optimized, the value was employed in subsequent optimization.

4.2.1 Type of Sorbent

C_{18}, C_8, C_2 and Porapak R sorbent materials were chosen to determine the sorbent material most suitable for use in the μ-SPE device. Apart from testing μ-SPE devices filled with purely C_{18}, C_8,

and C_2, the suitability of μ-SPE devices filled with various combinations of polar/non polar (1:1, 1.0 g of each) sorbent materials were also tested. The combinations tested were: C_{18} with Porapak R, C_8 with Porapak R, and C_2 with Porapak R. In total, six different types of sorbents were investigated. C_{18} has the highest hydrophobicity, followed by C_8 and then C_2. Porapak R, on the contrary, has intermediate polarity. It was selected to introduce certain degree of polarity. Extraction performances of the sorbents were evaluated by comparing extraction peak areas as shown in Figure 4.2.1. The μ-SPE device containing only C_{18} sorbent material was found to be most effective in extracting all target analytes, except dieldrin. Hence, sorbent material with the highest hydrophobicity was concluded to be most compatible with the target analytes.

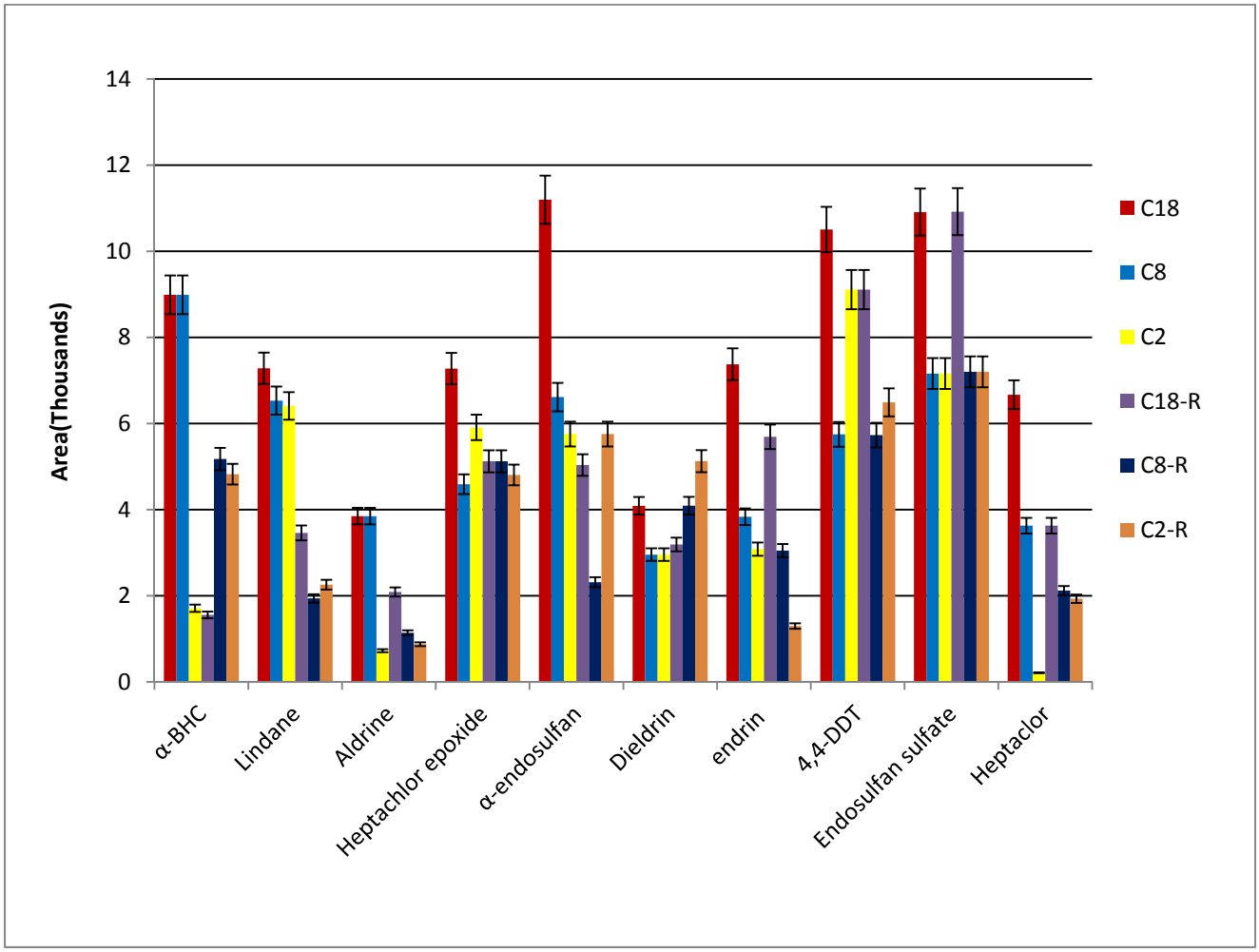


Figure 4.2.1.Effect of sorbent material on μ-SPE. Extraction conditions: Sorbent amount was 2 g, extraction time was 6 cycles (6.47 minutes per cycle), analytes were desorbed in 40 mL hexane by 40-minute ultrasonication

4.2.2 Desorption Solvent

It was essential to determine a suitable desorption solvent because the partition coefficient of a compound varies in different solvents. The solubility of a compound in a particular solvent depends on the very nature of the compound. Therefore, the desorption solvent has to have polarity as close as possible to that of the compound to boost extraction efficiency. Various organic solvents such as n-hexane, toluene, methanol, acetone, and dichloromethane were tested. As illustrated in Figure 4.2.2, polar solvents such as methanol and acetone were ineffective in desorbing the target analytes, giving substantially smaller peak areas. On the contrary, non-polar solvents such as n-hexane and toluene scored better results. This was explained by the fact that the OCPs are generally non-polar. n-Hexane was the best desorption solvent among the solvents tested.

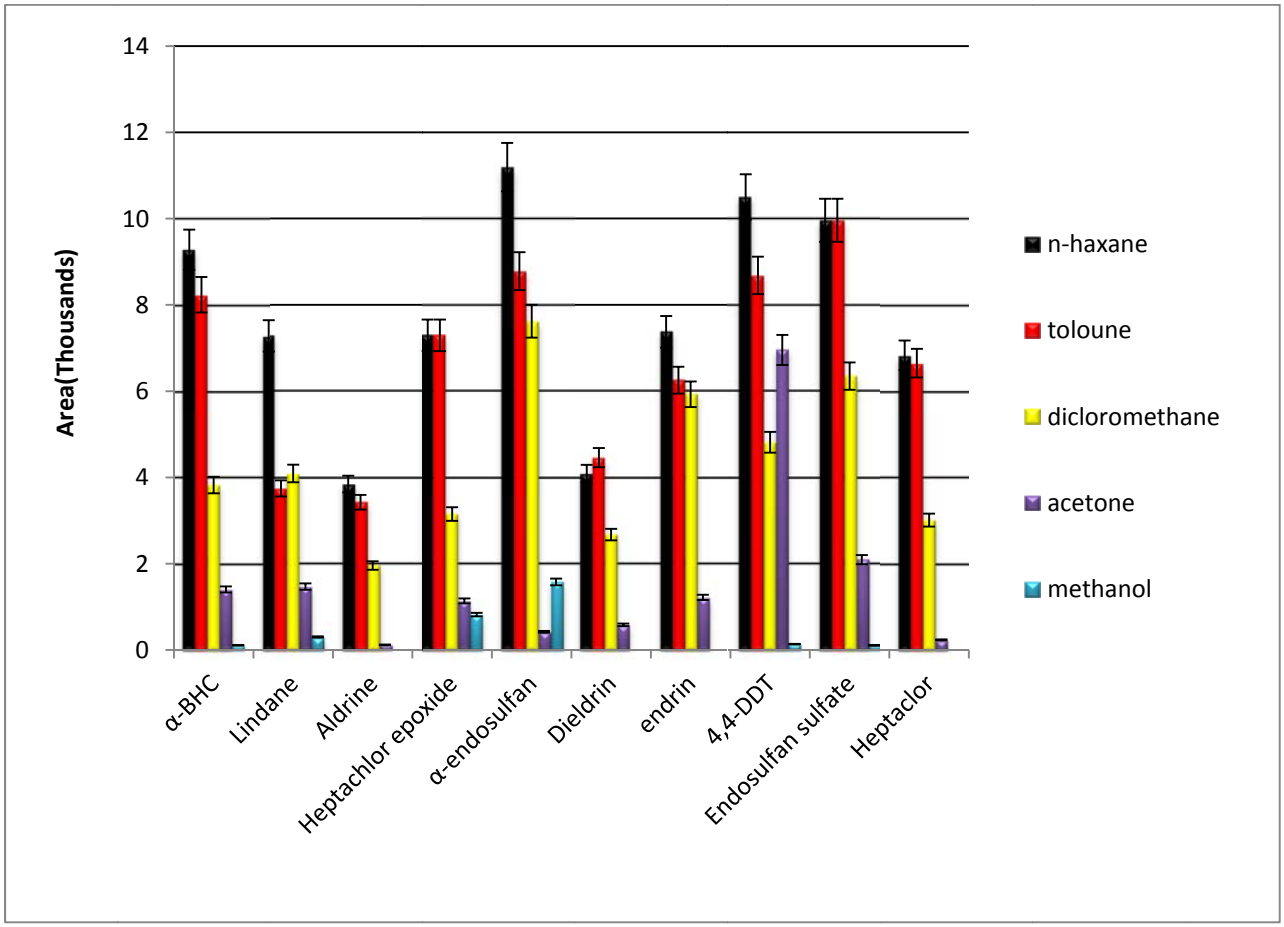


Figure 4.2.2. Effect of desorption solvent on μ-SPE. Extraction conditions: 2 g of C_{18} as sorbent material, extraction time was 6 cycles (6.47 minutes per cycle), analytes were desorbed in 40 mL solvent by 40-minute

4.2.3 Sorbent Amount

The impact of sorbent amount on extraction efficiency was investigated. The amount of sorbent material (2, 3, 4, and 5g) were used for the μ-SPE device operation was limited by the allowed size of the membrane envelope. The results from peak area analysis were illustrated in Figure 4.2.3, 2g and 4 g were found to be the two most effective sorbent amounts, being able to extract most of the target analytes. Therefore, 4g was chosen over 2g to be the optimized sorbent amount. When 4 g sorbent enhanced the extraction of certain target analytes, it did so to a large extent. On the contrary, in the case of 2 g sorbent, its performance was often only slightly better than 3 g and 4 g sorbents. It was observed that extraction efficiency was low when 5 g of sorbent was used for the μ-SPE device. This was because 5 g of sorbent filled the membrane envelope almost to the brim, greatly reducing the effective amount of sorbent material participating in the adsorption of target analytes.

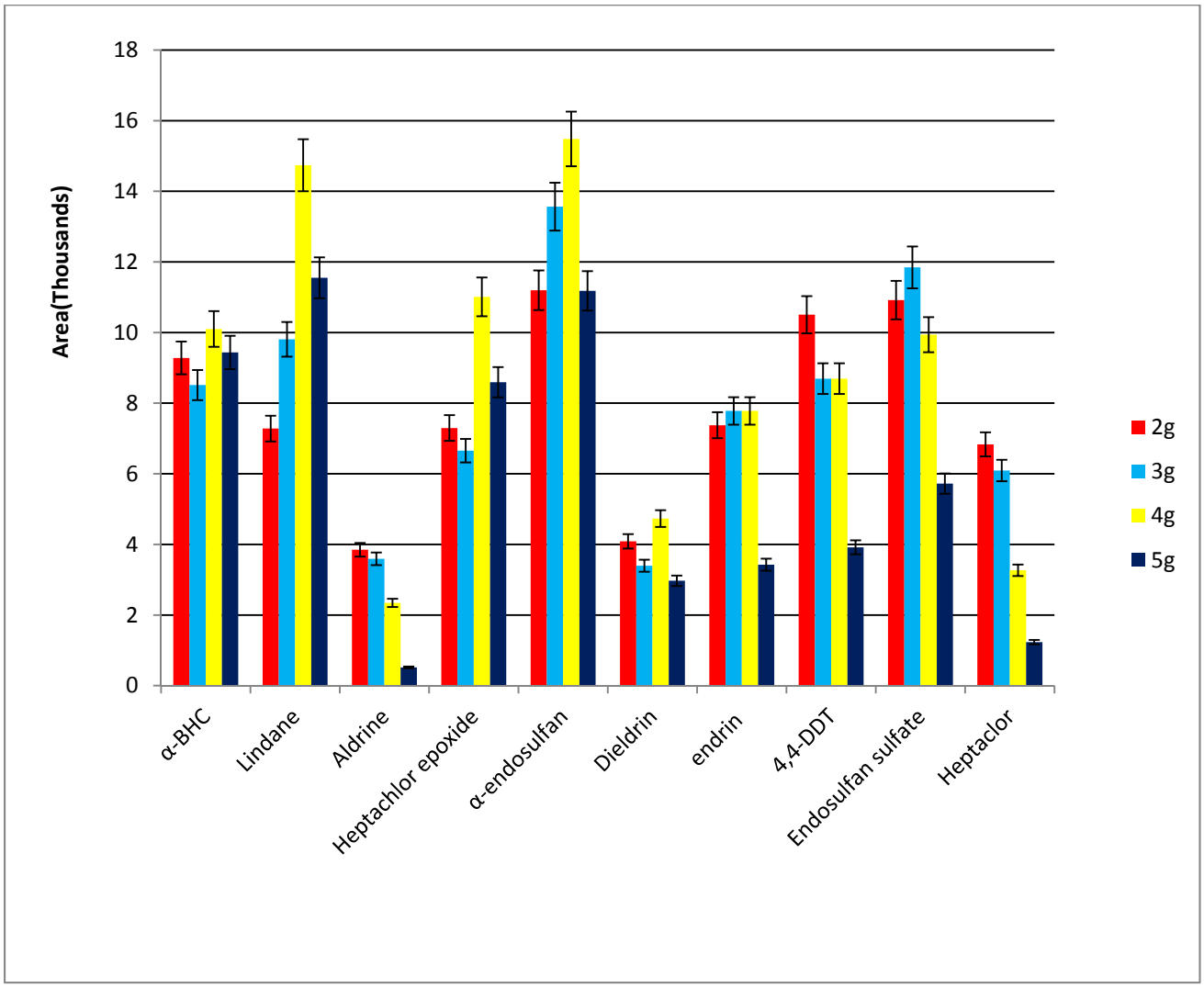


Figure 4.2.3 Effect of sorbent quantity on μ-SPE. Extraction conditions: C_{18} as sorbent material, extraction time was 6 cycles (6.47 minutes per cycle), analytes were desorbed in 40 mL n-hexane by 40-minute ultrasonication.

4.2.4 Extraction Time

The amount of analyte extracted depends on the mass transfer of analyte from the sample solution to the solid sorbent material. As mass transfer is dependent on time, the time required to maximize extraction efficiency was evaluated. The extraction time was calculated in terms of cycles. One cycle would run 10 L of spiked tap water, taking approximately 6.47 minutes. The longest extraction time tested was only 7 cycles. This was because it was one of the intentions to provide an efficient on-site sampling system but 7 cycles already took 45.29 minutes. Moreover, as depicted in Figure 4.2.4, the optimal number of cycles was found to be 6 which took 38.82 minutes. A longer extraction time only increased the amounts extracted for most of the target analytes and even so, the rise in analyte yield was not substantial. Therefore, 6 cycles with the extraction time of 38.82 minutes was determined to be the optimum extraction time.

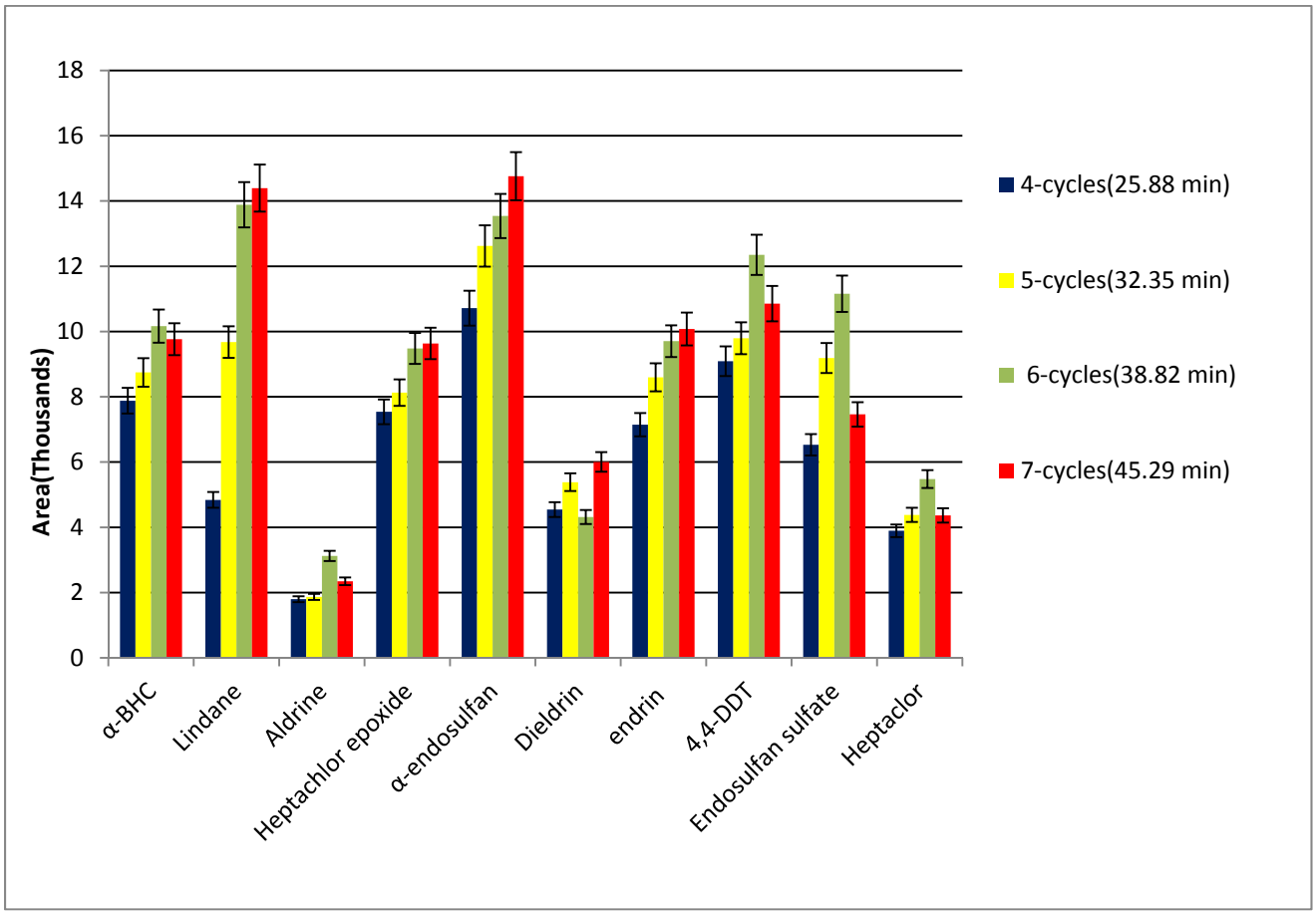


Figure 4.2.4 Effect of extraction time on μ-SPE. Extraction conditions: 4 g of C_{18} as sorbent material, analytes were desorbed in 40 mL n-hexane by 40-minute ultrasonication.

4.3 Extraction Method Evaluation

To appraise the feasibility of the proposed μ-SPE method, the optimized extraction conditions were employed to determine the following: the method's linearity, repeatability, limits of detection (LOD), limits of quantification (LOQ), enrichment factor and relative recovery. The linearity of the extraction method was tested at four concentration levels from 0.05 mg L^{-1} to 1.0 mg L^{-1}. Repeatability was investigated through triplicate analysis at various analyte concentrations within the linear range. Acceptable repeatability of relative standard deviation (RSD), which varied from 10.0 to 21.7%, was achieved. The LOD was calculated based on Sound to Noise ratio equals to 3 while the LOQ was determined by Sound to Noise ratio equals to 10. The data are summarized and shown in Table 4.3a. The percentage recovery for individual target analyte was calculated using the internal standard calibration method.

Analyte	Linearity (mg L^{-1})	RSD (%, n=3)	LOD (ng L^{-1}, n=3)	LOQ (ng L^{-1}, n=3)	Correlation Coefficient (r)
α-BHC	0.05 - 1.0	15.3	4	14	0.982
Heptachlor epoxide	0.05 - 1.0	10.0	2	5	0.973
Lindane	0.05 - 1.0	14.3	2	15.5	0.998
α-endosulfan	0.05 - 1.0	20.7	2	7.1	0.995
heptachlor	0.05 - 1.0	20.3	5	16.4	0.998
Aldrin	0.05 - 1.0	11.0	5	15.8	0.987
dieldrin	0.05 - 1.0	13.2	4	10.9	0.996
endrin	0.05 - 1.0	17.7	20	52.5	0.996
4,4-DDT	0.05 - 1.0	12.4	10	30	0.997
Endosulfan sulphate	0.05 - 1.0	14.5	3	9	0.968

Table 4.3 a. Method optimization data for OCPs.

Analyte	LLE Recovery (%, n=3)	μ-SPE Device Recovery (%, n=3)
α-BHC	79.2	89.2
Heptachlor epoxide	97.5	93.4
Lindane	93.9	94.8
α-endosulfan	93.5	95.1
Heptachlor	87.6	89.4
Aldrin	76.2	79.6
Dieldrin	89.1	92.3
Endrin	85.6	89.2
4,4-DDT	91.8	96.2
Endosulfan sulphate	115.3	89.6

Table 4.3 b. Percentage recovery data for OCPs.

4.4 Acceptable intake levels of OCPs in water and sea foods

Various monitoring agencies have different acceptable OCPs levels. Each agency having its own standards based on the level of contamination of the environment with different pesticides, below is table showing the various levels from different monitoring bodies.

OCPs analytes	Acceptable intake levels in drinking water (μg/l)	Acceptable daily intake in Fish (mg/kg)
α-BHC	2[3]	0.4[3]
Heptachlor epoxide	0.03[3]	0.3[1]
Lindane	2[3]	0.4[3]
α-endosulfan	6 ug/kg	0.05
Heptachlor	0.03[3]	0.3[1]
Aldrin	0.03 [3]	0.3[2]
Dieldrin	0.3[4]	0.3[4]
Endrin	2[1]	0.3[2]
p,p'-DDT	2[3]	5[2]
Endosulfan sulphate	0.05 [5]	0.05 μg /g[5]

Table 4.4 Acceptable OCPs levels in drinking water and fish daily intake

Note

1) US EPA 1990
2) National standards and guidelines for pesticides review Germany vol 140
3) WHO 1993 Vol 1
4) Australian and New Zealand Environment and Conservation Council
5) European union standards

4.5 Real Sample Analysis

The optimized μ-SPE method was applied to on-site extraction of the target analytes from sea water. The on-site sampling was performed at on various sites of eastern province of the Kingdom of Saudi Arabia. Care was taken to extract all water samples from a depth of 0.45 m to avoid contamination from the surface micro-layer. The sampling system was run for 38.82 minutes before the μ-SPE device was removed and handled with the extraction procedure described earlier.

SAMPLE	*α-BHC*	*LINDANE*	*HEPTACHLOR*	*ALDRIN -R*	*HEPTACHLOR EPOXIDE*	α- *ENDOSULFAN*	*DIELDRIN*	*4,4- DDT*	*ENDRIN*	*ENDOSULFAN SULFATE*
AG 1	0.074	0.940	Nd	Nd	Nd	0.010	Nd	Nd	Nd	Nd
AG 2	0.139	1.595	Nd	Nd	Nd	0.023	Nd	Nd	Nd	Nd
AG 3	0.010	0.092	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
AG 4	0.021	0.169	Nd	Nd	Nd	0.033	Nd	Nd	Nd	Nd
AG 5	0.015	0.082	Nd	Nd	Nd	0.004	Nd	Nd	Nd	Nd
AG 6	0.048	0.125	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
AG 7	0.113	0.179	Nd	0.067	Nd	Nd	Nd	Nd	Nd	Nd
AG 8	0.033	Nd	Nd	Nd	Nd	0.005	Nd	Nd	Nd	Nd
AG 9	0.218	0.295	Nd	Nd	Nd	0.012	Nd	Nd	Nd	0.945
AG 10	Nd	0.188	Nd	Nd	Nd	Nd	Nd	Nd	11.71	Nd
AG 11	0.177	0.180	Nd	Nd	Nd	Nd	Nd	Nd	11.71	Nd
AG 12	0.033	0.129	Nd	Nd	Nd	Nd	Nd	Nd	nd	Nd
AG 13	0.036	0.314	Nd	Nd	Nd	Nd		Nd	nd	Nd
AG 14	0.332	0.160	Nd	0.016	Nd	0.057	0.391	Nd	3.206	Nd

Table 4.5 b Concentration of OCPS in Seawater samples . (μg L^{-1})

Nd: Not detected

Target analyte	Mean of OCPS(n=3)
α-BHC	0.0952
Lindane	0.3420
Aldrine	0.0415
α-endosulfan	0.0203
Endrin	7.456

Table 4.5 c Mean concentration of OCPs (μg L^{-1})

To obtain a more accurate assessment of the proposed method's extraction efficiency, the μ-SPE method was compared to Liquid Liquid Extraction (LLE). LLE is an exhaustive extraction method used to monitor OCPs in water. 3 L of sea water was collected to prepare 3 samples. The sea water used for LLE was sampled from the same depth as that used for μ-SPE. This was done so by employing the pump of the sampling system. The sea water was collected into one 1 L glass bottle and one 2 L glass bottle, both of which were preserved with 5 mL and 10 mL of n-hexane respectively. This was intended to avoid the loss of volatile organic compounds [24]. The glass bottles were wrapped with aluminium foil to keep away sunlight and were stored in ice during transportation to the lab. Analysis was performed on the same day of sampling. The extraction procedure followed the Standard method of analyzing OCPs in water [20].

The levels of OCPs detected in sea water using the two different methods were compared. It was observed that the amounts extracted by μ-SPE were comparable to those extracted by LLE for all target analytes.

Percentage recovery of Liquid-liquid extraction and μ-SPE method were compared in the table 4.5d.

Analyte	LLE recovery (% n=3)	μ-SPE device recovery (% n=3)
α-BHC	79.2	89.2
Lindane	97.5	93.4
Heptachlor	93.9	94.8
Heptachlor epoxide	93.5	95.1
α -endosulfan	87.6	89.4
Aldrin	76.2	79.6
Dieldrin	89.1	92.3
4,4 DDT	85.6	89.2
Endrin	91.8	96.2
Endosulfan sulfate	115.3	89.6

Table 4.5d Comparison of recoveries between LLE and μ-SPE

RESULTS AND DISCUSSION

PART II: ANALYSIS OF OCPs IN SEAFOOD

Seafood samples were extracted using well established method.before quantitation GC/MS conditions and calibrations were performed. Figure 4.3 shows Standard chromatogram of 250 ng/g concentration of target OCPs

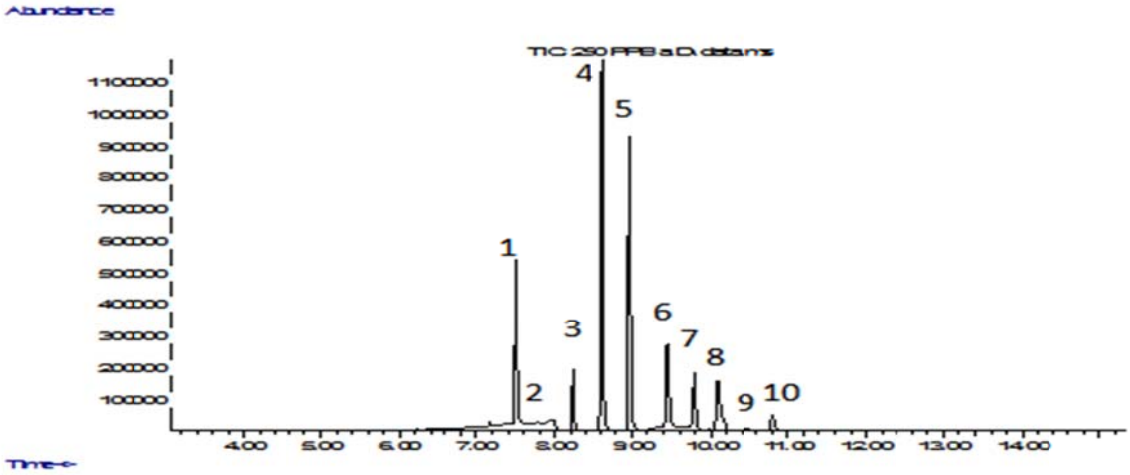


Fig 4.3: GC/MS chromatogram of OCP standard analytes

OCPS

1: α-BHC

2: Lindane

3: Heptachlor

4: Aldrin-R

5: Heptachlor epoxide

6: α-Endosulfan

7: Dieldrin

8: 4,4 DDT

9: Endrin

10: Endosulfan sulfate

4.6 Calibration

Calibration graphs were established through the range of 0.05-1 ng/g below are the calibration curves for the standard solution of each target analyte:

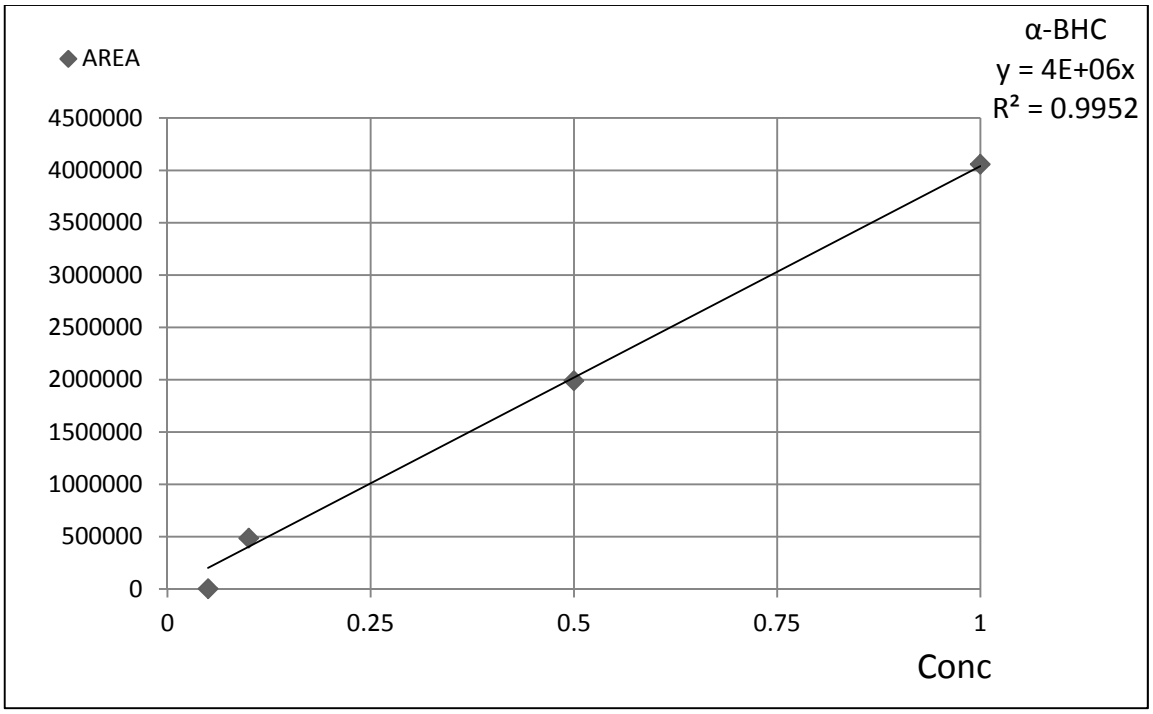


Fig 4.6 a calibration curve for α -BHC analyte

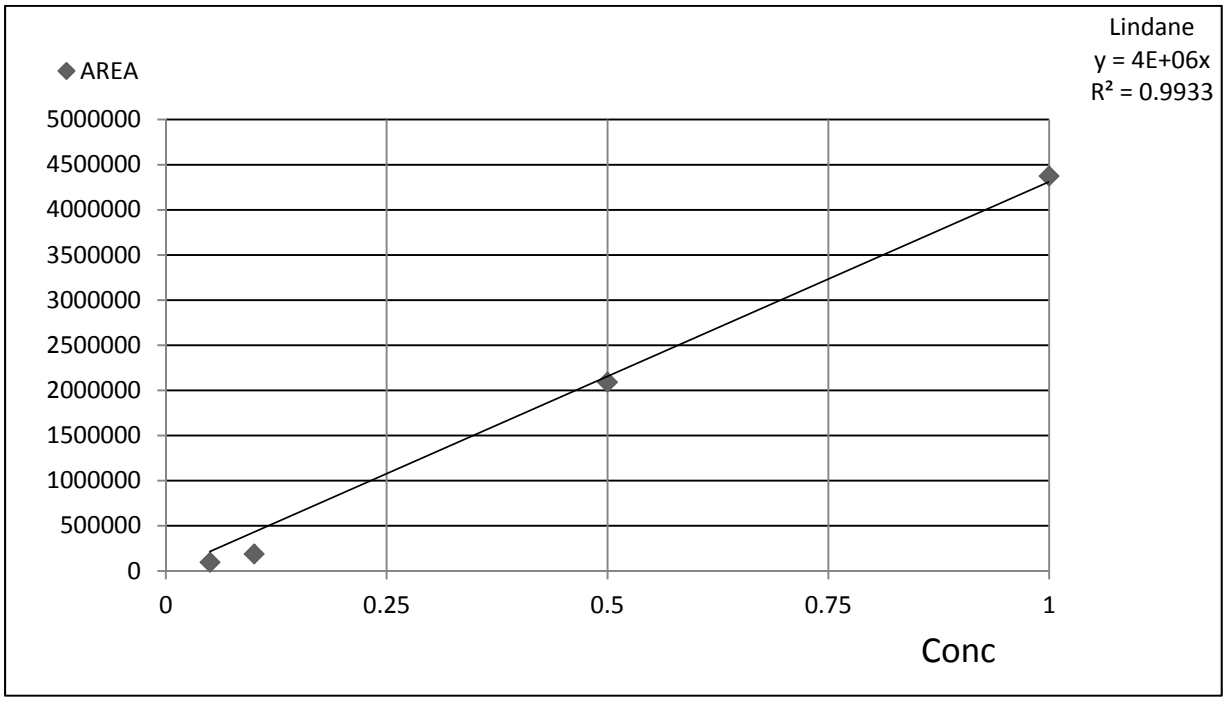


Fig 4.6b calibration curve for Lindane analyte

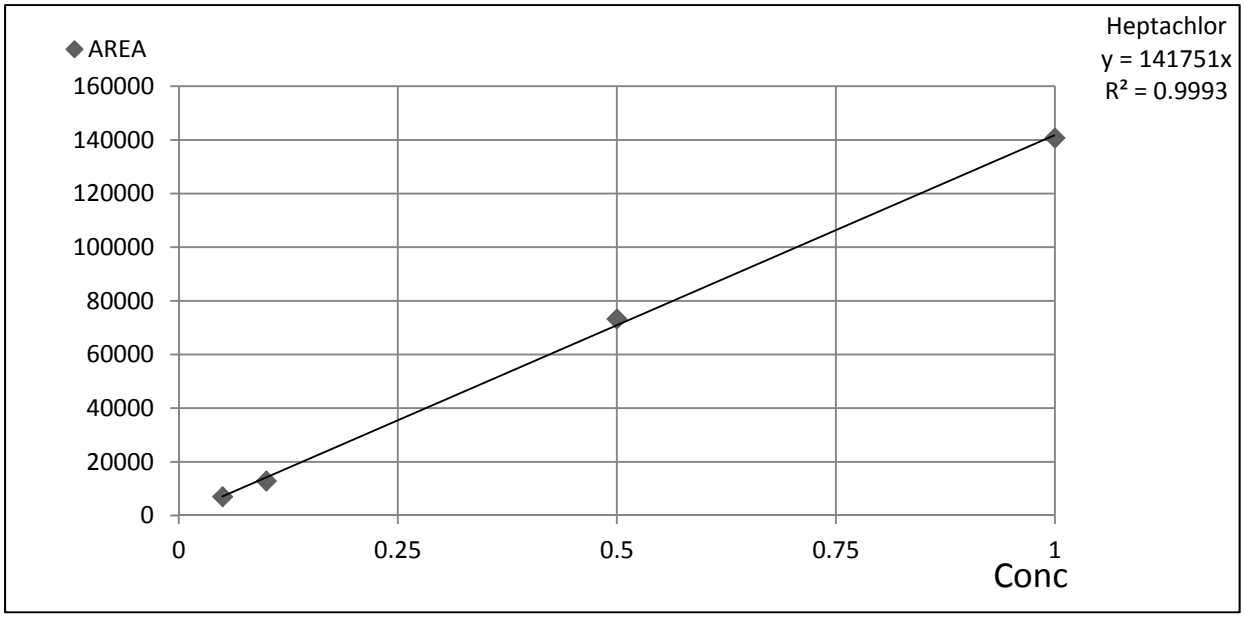


Fig 4.6c calibration curve for heptachlor analyte

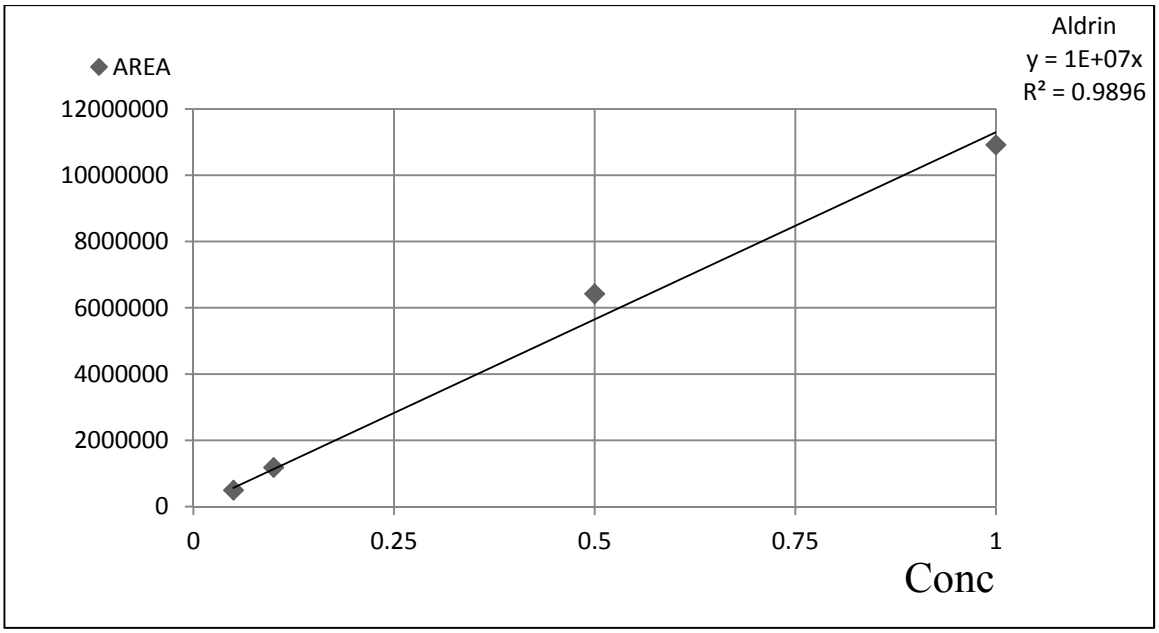


Fig 4.6d calibration curve for Aldrin analyte

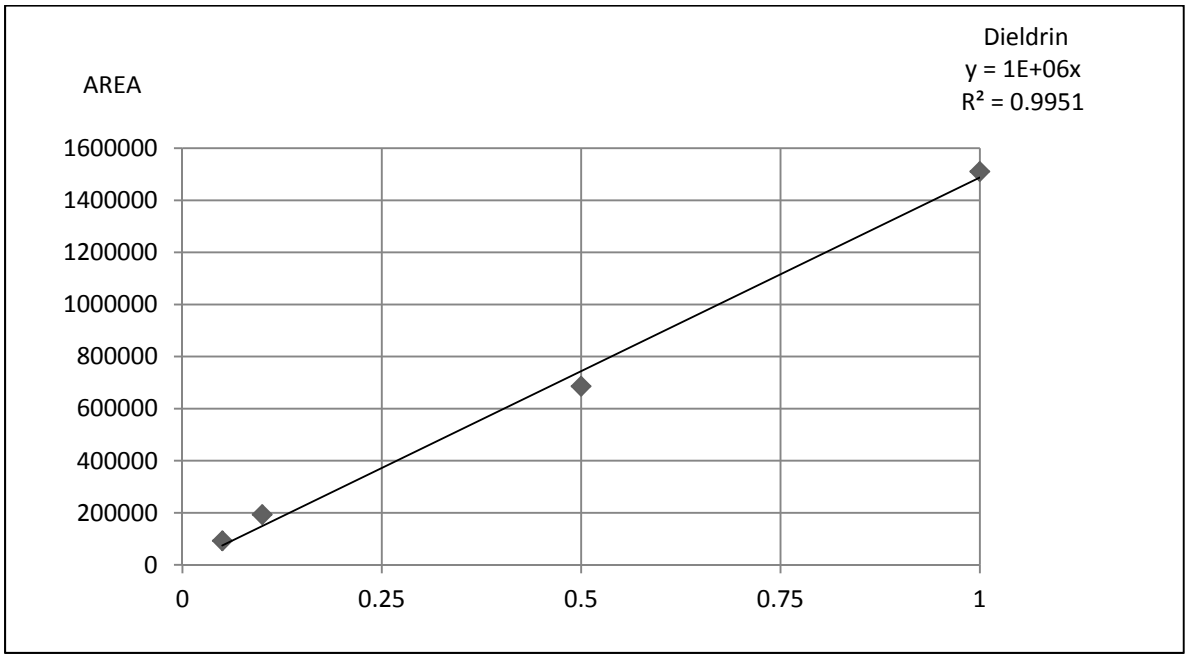


Fig 4.6e calibration curve for Dieldrin analyte

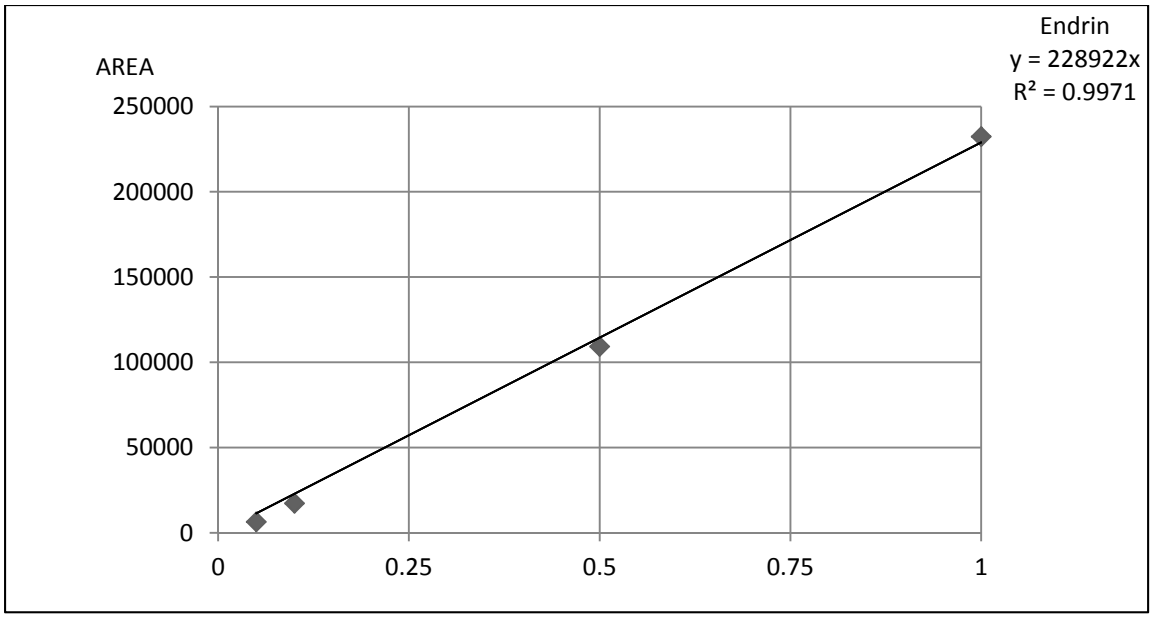


Fig 4.6f calibration curve for Endrin analyte

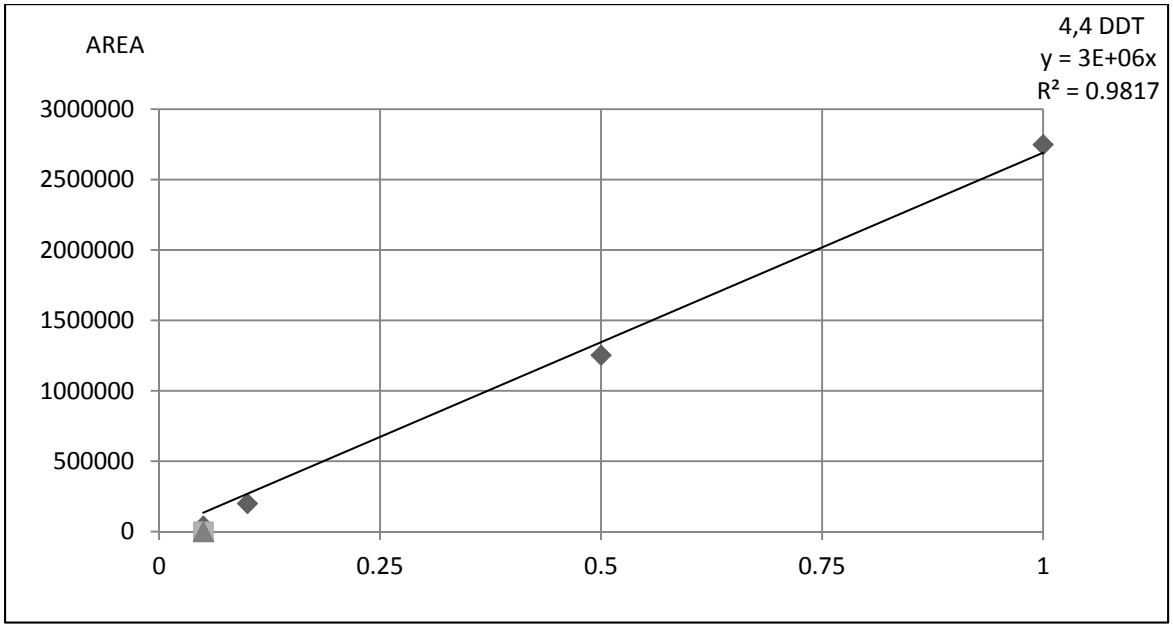


Fig 4.6g Calibration curve for 4,4 DDT analyte

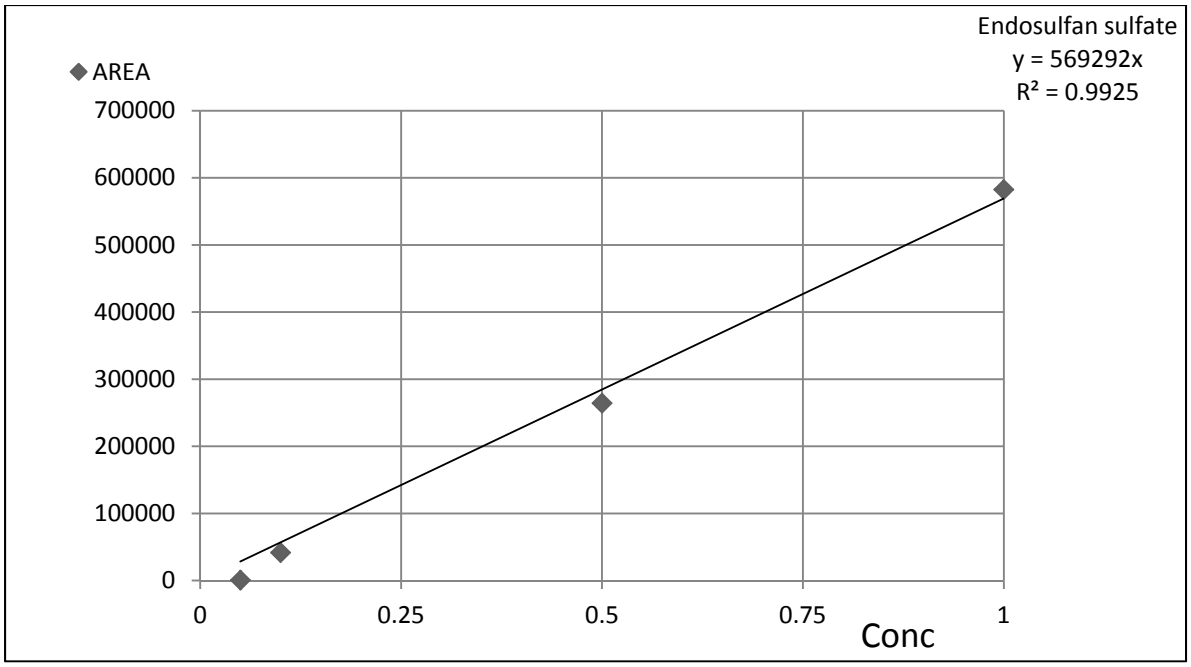


Fig 4.4h Calibration curve for Endosulfan Sulfate analyte

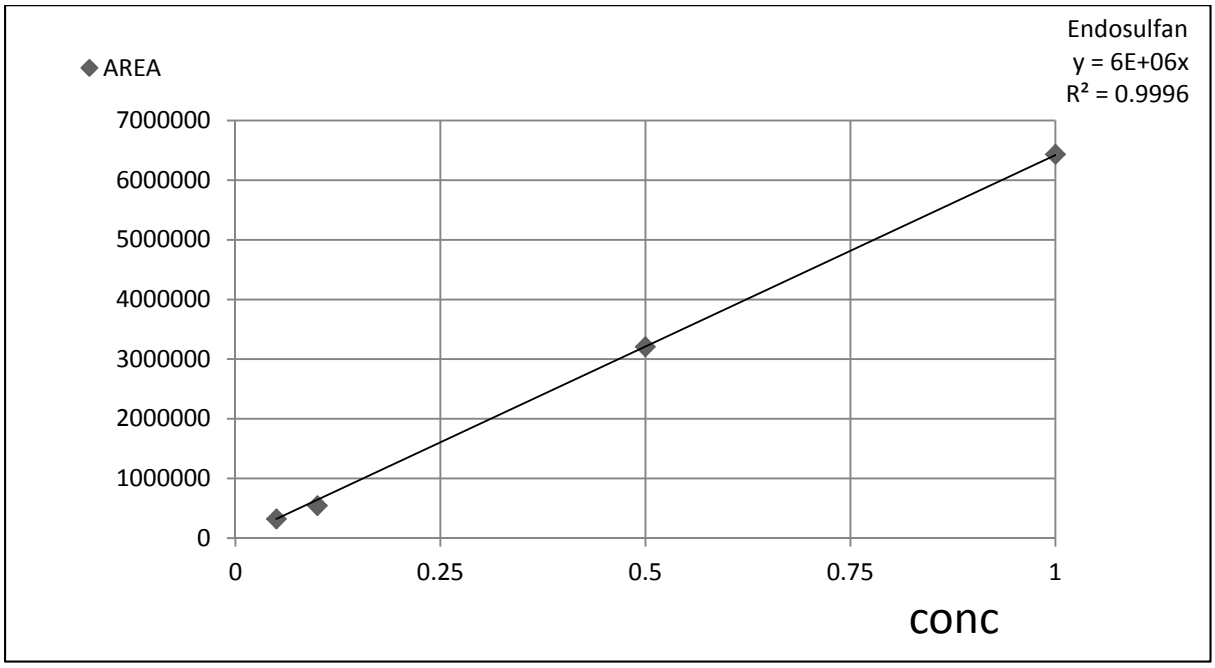


Fig 4.4i Calibration curve for α –Endosulfan analyte

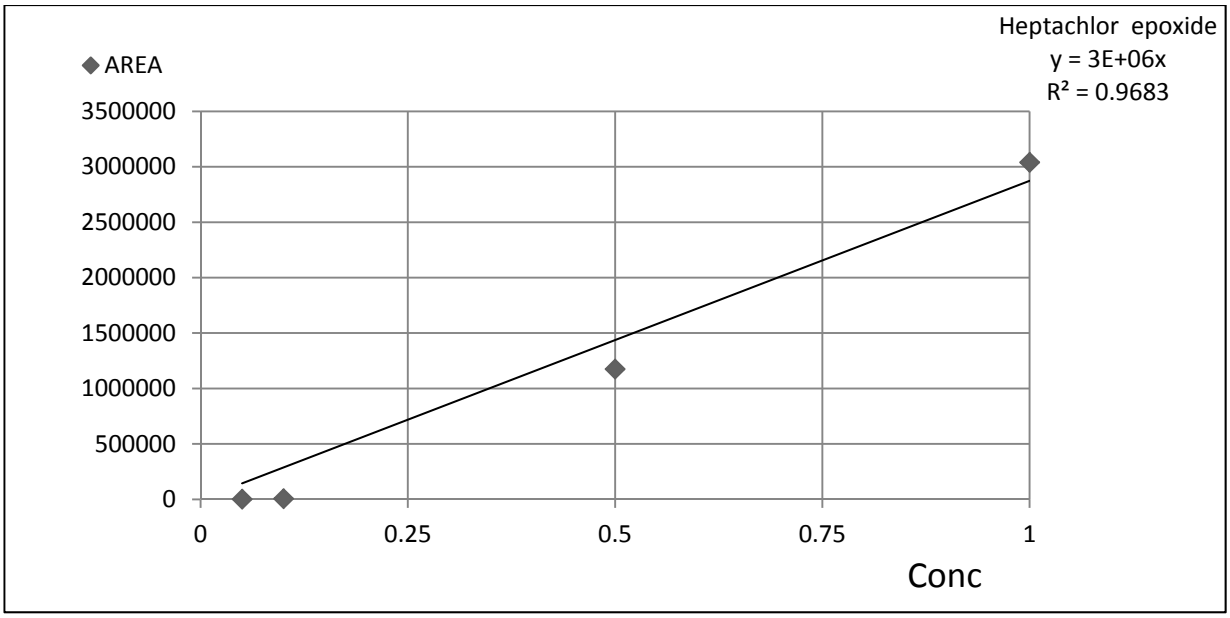


Fig 4.4j Calibration curve for heptachlor epoxide analyte

4.7 Percentage recovery

Recovery of OCPs target pesticide validation was carried out by spiking 200 ng/g of standard into 2g liver of a squid and extraction process was conducted and then analysis done.

	% Recovery					
	S_1	S_2	S_3	S_4	S_5	Avg
α-BHC	94.8	87.2	97.7	92.7	96.3	93.7
Lindane	95.8	95.5	97.7	99.5	88.5	95.4
Heptachlor	97.4	99.6	85.8	96.6	85.6	93.0
Heptachlor epoxide	89.7	89.8	84.4	85.2	86.6	87.2
α -endosulfan	89.3	95.6	98.6	90.1	86.5	92.0
Aldrin	86.6	93.7	91.7	85.8	94.9	90.5
Dieldrin	94.1	85.2	91.6	85.2	91.0	89.4
4,4 DDT	93.9	85.7	93.5	88.0	91.6	90.6
Endrin	88.8	85.9	83.2	84.6	84.0	85.3
Endosulfan sulfate	81.9	53.2	82.7	82.4	83.1	76.7

Table 4.7 Extraction recoveries of OCPs in seafood sample

Each sample was analyzed for the target OCPs below is a chromatogram for one of the extracted sample

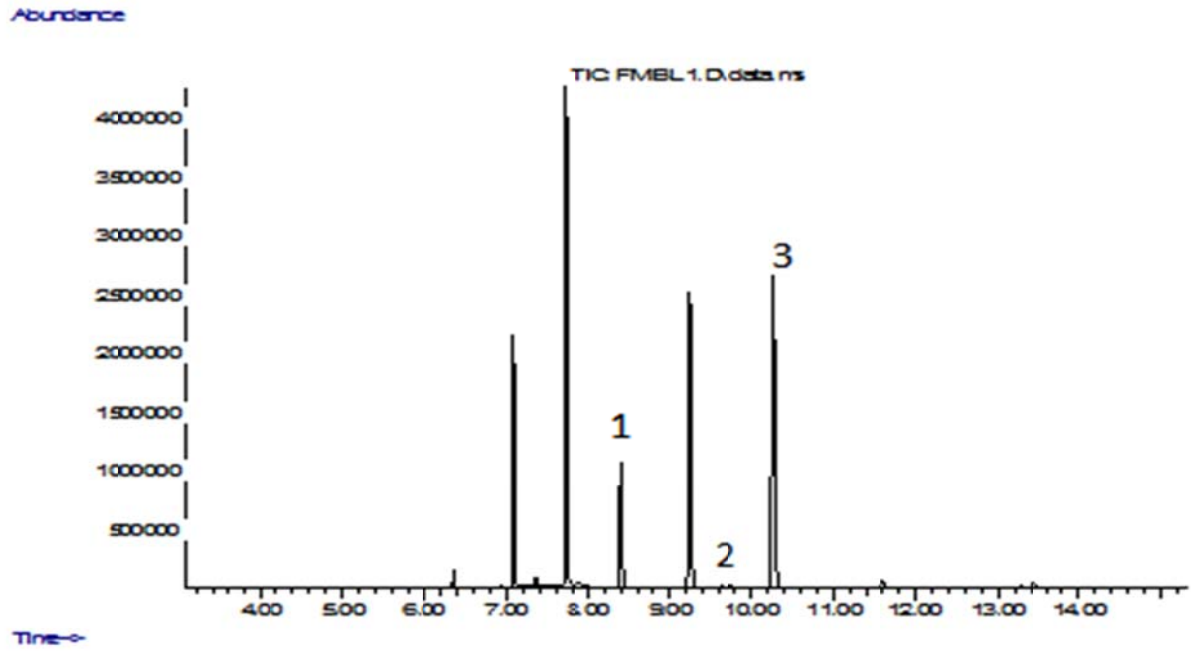


Fig 4.7: GC/MS chromatogram of fresh clamp mussels showing OCPs peaks detected

1: Heptachlor 2: Dieldrin 3: Endrin

4.8 Sea food analysis

The following seafood samples we collected and their names were abbreviated in the table 4.8.1. after extraction of OCPs concentration were calculated using previously established calibration.

Samples	Abbreviations	Samples	Abbreviations
NAJIIL DAMMAM/LIVER	*NJ/L*	*FRESH MUSCLE-ABU ALI(S)/LIVER BODY*	*AA/FM/BL*
NAJIIL DAMMAN/BODY	*NJ/B*	*FRESH MUSCLE/BODY/LIVER -2*	*FM/BL-2*
HARRID PARROT/LIVER	*HP/L*	*FASKER FISH/LIVER-2*	*FK/L-2*
HARRID PARROT/BODY	*HP/B*	*FASKER FISH/BODY-2*	*FK/B-2*
FASKER FISH/LIVER	*FK/L*	*SHRIMPS/BODY LIVER-2*	*S/BL-2*
FASKER FISH/BODY	*FK/B*	*RED SQIUD/LIVER-2*	*RS/L-2*
RED SQIUD/LIVER	*RS/L*	*RED SQIUD/BODY-2*	*RS/B-2*
RED SQIUD/BODY	*RS/B*	*MALE CRAB/BODY/LIVER-2*	*MC/BL-2*
SHRIMPS/BODY LIVER	*S/BL*	*NAJIIL DAMMAM/LIVER*	*NJ/L-2*
FEMALE CRAB/BODY/LIVER	*FC/BL*	*NAJIIL DAMMAN/BODY*	*NJ/B-2*
MALE CRAB/BODY/LIVER	*MC/BL*	*FRESH MUSCLE-ABU ALI(N)/LIVER/BODY*	*FM/AN/BL*
FRESH MUSCLE/BODY LIVER	*FM/BL*	*FRESH MUSCLE-MANIFA/LIVER BODY*	*FM/M/BL*
FRESH MUSCLE TARUS/BODY/LIVER	*FM/T/BL*	*FRESH MUSCLE-SAFANIYA/LIVER BODY*	*FM/S/BL*
CRAB-ABU ALI(S)/LIVER/BODY	*AA/C/BL*		

Table 4.8.1: Samples analyzed and their abbreviation

4.8.1 Supermarket samples concentration

SAMPLE	*α-BHC*	*LINDANE*	*HEPTACHLOR*	*ALDRIN – R*	*HEPTACHLOR EPOXIDE*	*ENDOSULFAN*	*DIELDRIN*	*4,4-DDT*	*ENDRIN*	*ENDOSULFAN SULFATE*
NJ/L	0.004	0.005	-	-	-	-	-	-	1.100	-
NJ/B	-	-	-	-	-	-	-	-	0.230	-
HP/L	-	0.007	-	-	-	-	-	-	-	-
HP/B	-	0.002		-	-	0.004	0.006	-	1.650	-
FK/L	-	-	0.346	-	-	-	-	-	2.195	-
FK/B	-	-	1.730	-	-	-	-	-	0.732	-
RS/L	-	-	3.460	-	-	-	-	-	27.44	-
RS/B	-	-	3.460	-	-	-	-	-	14.63	-
S/BL	-	0.002	0.732	-	0.002	-	0.005	-	0.7	
FC/BL	-	-	-	-		-	0.200	-	12.80	0.794
MC/BL	-	0.001	0.011	-	-	-	0.002	-	0.184	-
FM/BL	-	-	2.422	-	0.011	-	0.420	-	23.78	-
MC/BL-2	-	0.375	0.025	0.005	0.005	0.070	-	-	-	0.015
NJ/L-2	-	0.288	0.220	0.018	-	-	-	-	-	0.151
NJ/B-2	-	0.500	0.018	0.003	-	0.014	-	-	-	0.040
FM/BL-2	0.004	0.003	-	-	-	-	-	-	0.073	-
FK/L-2	-	0.150		0.006	-	-	-	-	0.366	0.172
FK/B-2	-	0.175	0.083	-	0.010	0.010		-	0.915	0.089
S/BL-2	-	0.038	0.012	-	0.002	-	0.090	-	0.298	0.044
RS/L-2	-	0.250	0.885	0.040	0.014	0.200	-	-	-	0.156
RS/B-2	-	0.010	-	0.005	0.007	0.010	-	-	0.180	-

Table 4.8.2 Concentration of OCPS in body and liver of sea foods.(ng g^{-1})-(SUPERMARKET)

4.8.2 Local market samples OCPs concentration

Sample	*α-BHC*	*LINDANE*	*HEPTACHLOR*	*ALDRIN*	*HEPTACHLOR EPOXIDE*	*ENDOSULFAN*	*DIELDRIN*	*4,4-DDT*	*ENDRIN*	*ENDOSULFAN SULFATE*
FM/T/BL	-	0.010	-	-	0.005	-	-		-	0.051
AA/C/BL	0.010	0.003	0.017	-	-	-	-		1.832	2.727
AA/FM/BL	0.010	0.003	0.016	-	-	0.002	0.009		0.549	-
FM/AN/BL	-	0.038	0.034	0.002	-	-	-	0.027	-	0.034
FM/M/BL	0.004	0.013	0.010	0.001	-	0.013	-	-	-	0.004
FM/S/BL	-	0.150	0.005	0.010	-	0.030	-	-	0.255	0.067

Table 4.8.3 Concentration of OCPs in body and liver of seafoods(local market) ng g^{-1}

Target analyte	Average amount detected in sea foods (ng g^{-1}, n=27)	Acceptable Daily Intake* (60 kg wt)
α-BHC	0.004	480µg
Lindane	0.101	480µg
Heptachlor	0.642	30µg
Aldrine	0.006	6µg
Heptachlor epoxide	0.006	1.76 (ng/kg body weight/day)
α-endosulfan	0.027	No data available
Dieldrin	0.092	6µg
Endrin	4.496	14.4 ng/g wgt per day[1]
4,4-DDT	0.014	20 ng/kg body wgt per day
Endosulfan sulphate	0.310	50 ng/g wgt per day[2]

Table 4.8.4: Mean amount of OCPs detected in the sea foods and acceptable daily intake per day

Note:

Any quantity below the Qualitative Limit is considered not detected.

"ND" = "not detected"

* Set by the WHO

(1) U.S Environmental protection Agency

(2) European union standards

4.8.3 Comparison of target analytes concentration in body and liver of different seafood

Comparison of OCP analyte in the liver and body of different sea food species

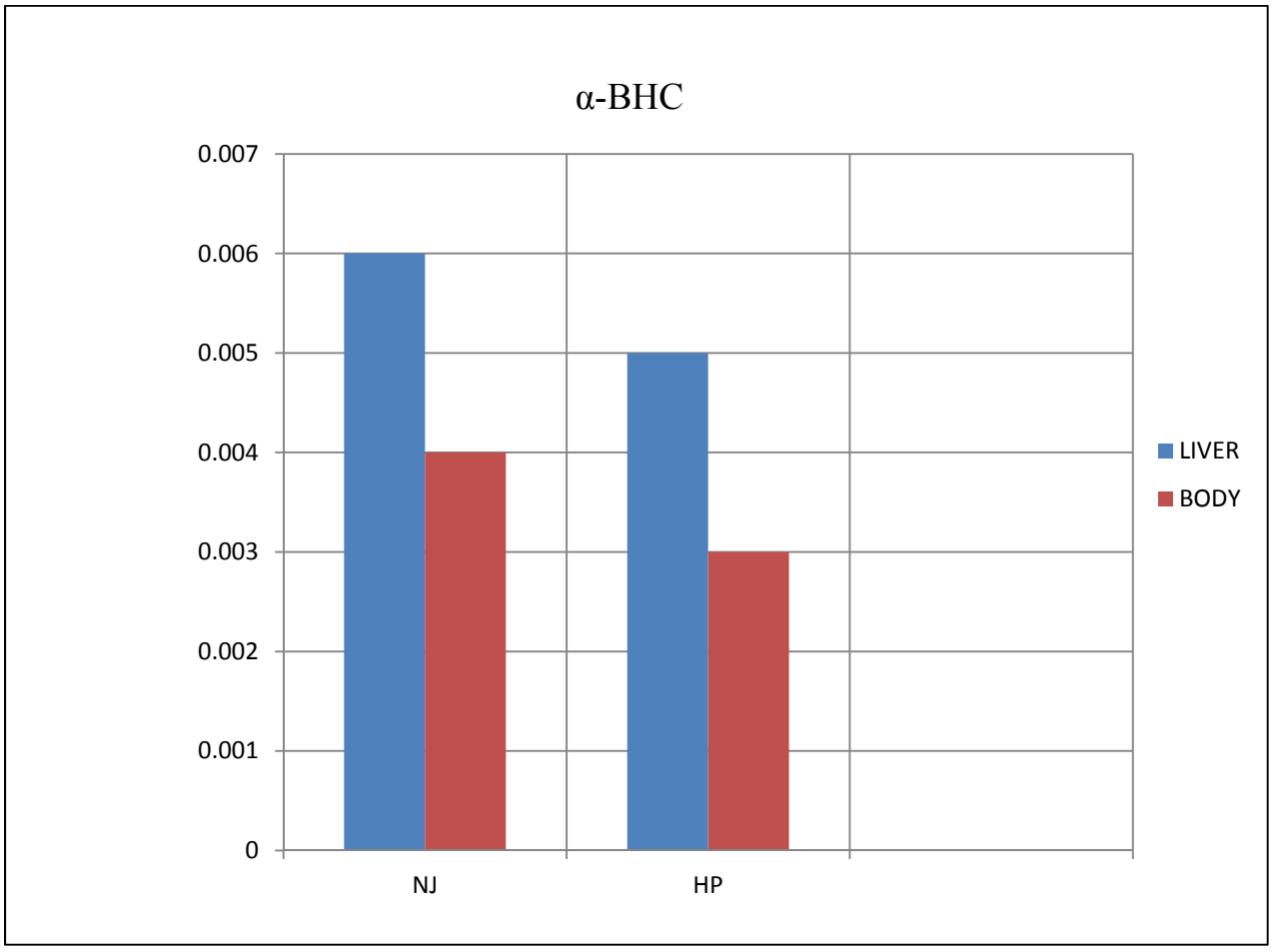


Fig 4.8a Comparison of α-BHC in liver and body of different samples

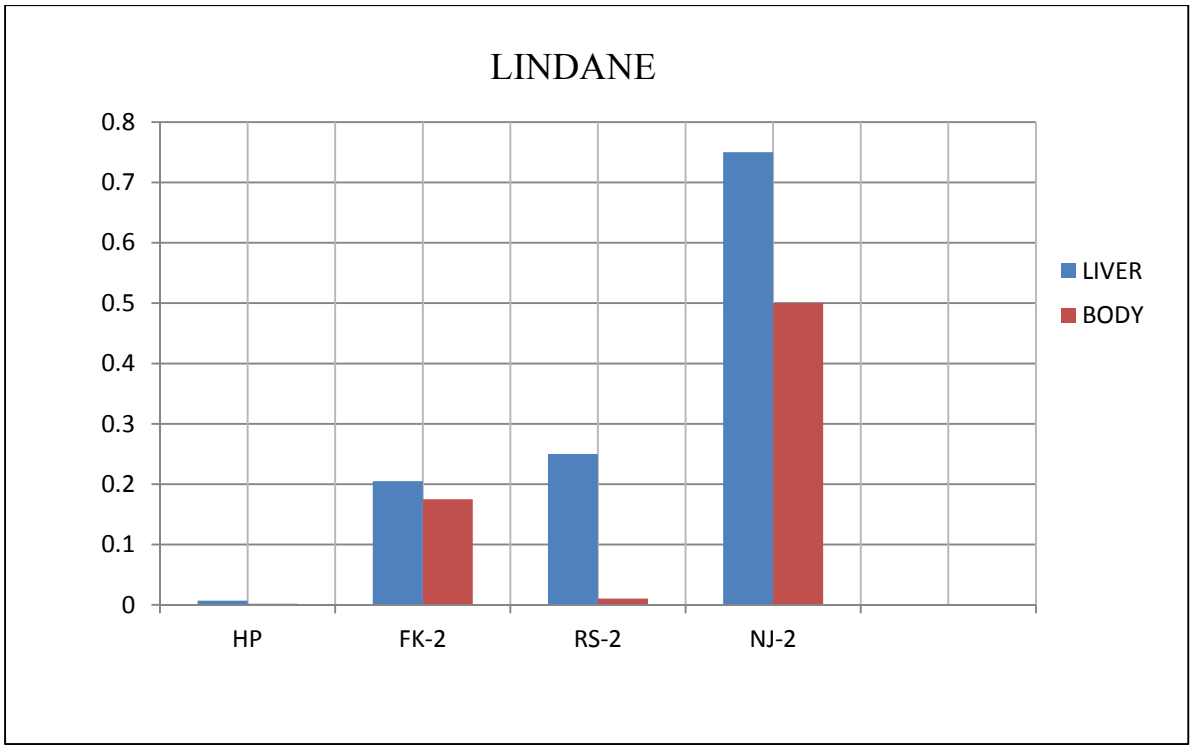


Fig 4.8b Comparison of Lindane in liver and body of different samples

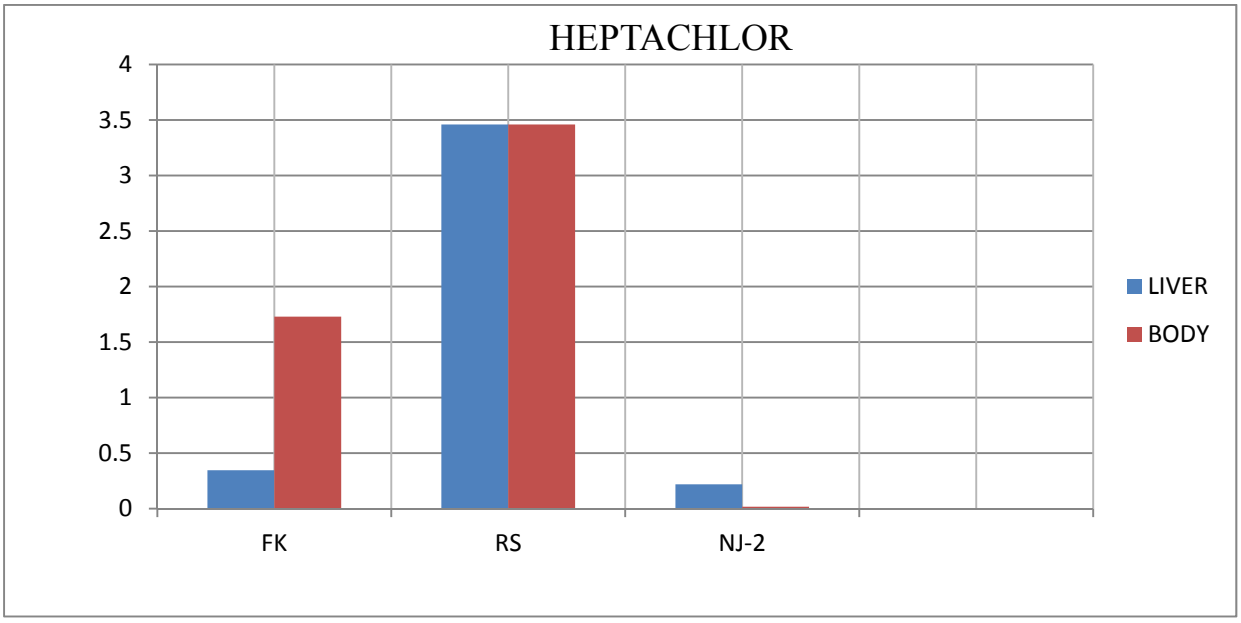


Fig 4.8c Comparison of Heptachlor in liver and body of different samples

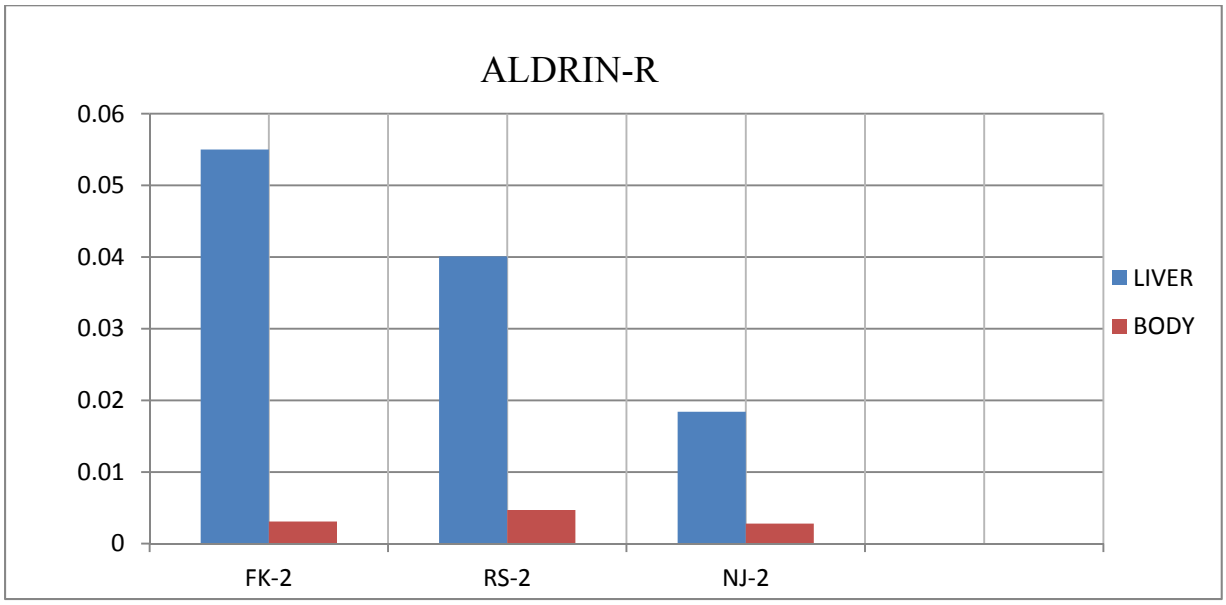


Fig 4.8d Comparison of Aldrin in liver and body of different samples

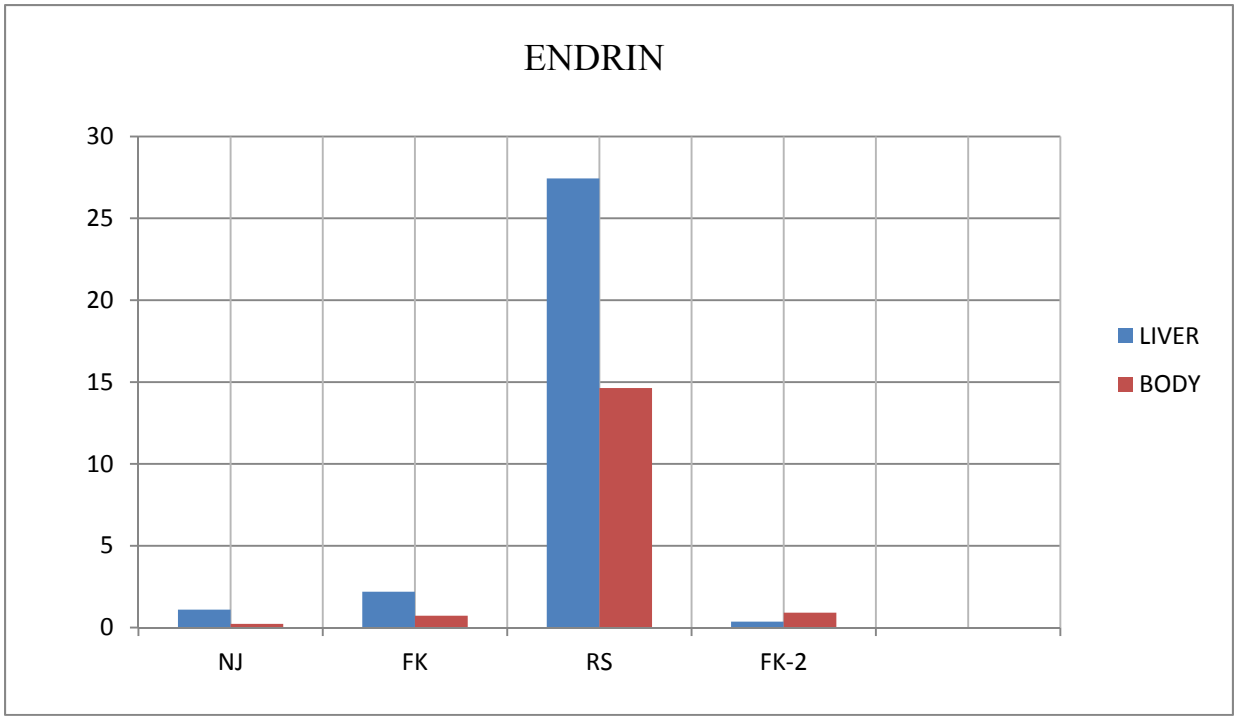


Fig 4.8e Comparison of Endrin in liver and body of different samples

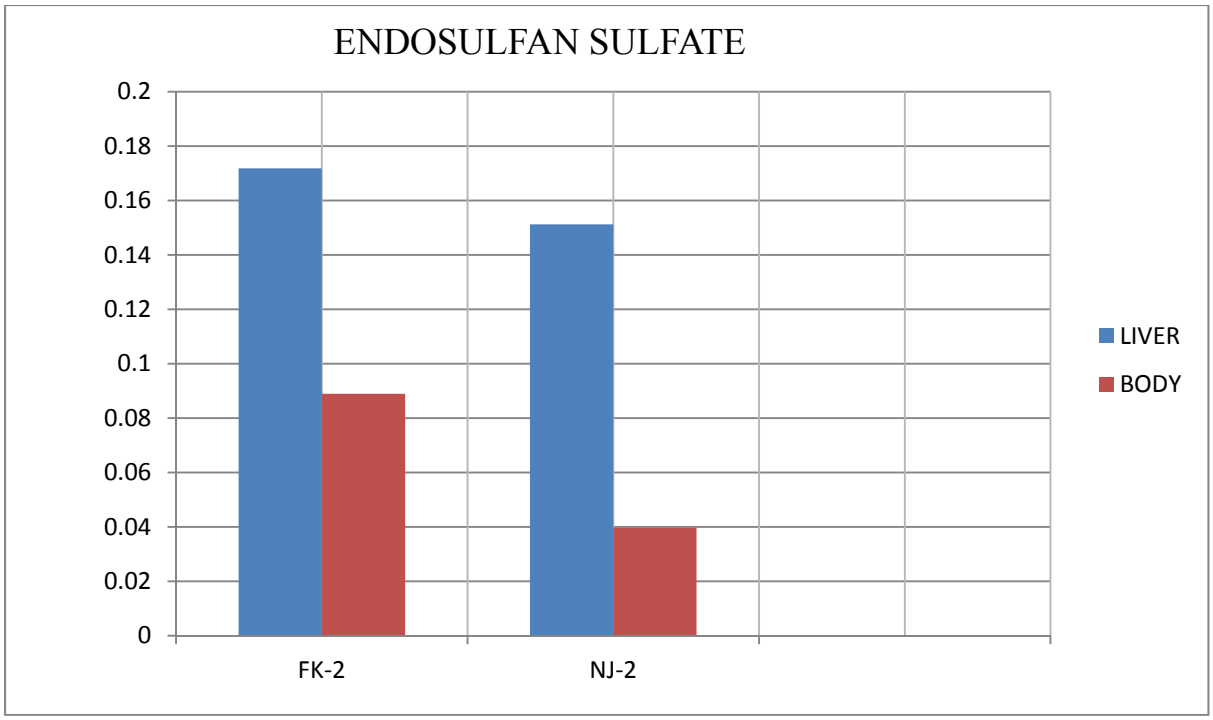


Fig 4.8f Comparison of Endosulfan sulphate in liver and body of different samples

4.9 DISCUSSION

Organochlorines Pesticides is a class of pesticides that have been prohibited worldwide since the beginning of 1980s due to their toxicity, stability, high liposolubility, long biological half-life, and consequently a high degree of bioaccumulation in food chain. Moreover, they are persistent in the environment and tend to accumulate in ecosystems. In the past extremely use of OCPs poses still dangerous effects such as cancer, immune systems, reduced bone mineral density, and the disruption of hormonal functions on health of animals, human and environment. Despite a prohibition imposed by WHO on use some of OCPs are still used in limited quantity in many developing countries.

The developed onsite sampling device proved a viable instrument that can be used to effectively monitor OCPs in sea water and any other water bodies. Selectivity and efficiency of different parameters that affects the use of the device were determined by performing optimization of different parameters as shown in the above discussion where type of sorbent, amount of sorbent, desorption solvent, and extraction time were optimized successfully. Then real analysis of the sea water in different location along the Arabian Gulf was done. The detected concentration portrayed that use of the pesticides still exists and the highest amount of 11.706 μg L^{-1} Endrin were noted at AG-10 and AG-11 sites, while lowest concentration was at AG-5 AND AG-8 sites of α-endosulfan. The concentration of target analytes in the area of study was below the WHO standards although a substantial presence of Endrin was noted in the Dammam area of study which shows most likely usage or persistence at the time in the environment.

The number of sea food samples analysed for the OCPs were 27 samples of different species. The highest concentration was detected in sample FM/BL (fresh muscles (body/liver)) with analyte Endrin having a concentration of 27.78 ng g^{-1} while the lowest concentration limit detected for the sample was Aldrin with a concentration of 0.033 ng g^{-1}.The highest mean concentration among the analytes was also with Endrin having 4.5 ng g^{-1} in 27 samples. The lowest mean was for α-BHC analyte of 0.004 ng g^{-1}.The comparison of concentration of target OCP analyte showed that it has high concentration in the liver than the body tissue. The detected amount is below the recommended daily intake by WHO and other agencies but due to the bioaccumulation of OCPs in the tissue and lipids of sea foods raises concern on its effect in the long term period.

CHAPTER 5: CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

Monitoring of the widespread distribution of pesticides in water requires the availability of fast, efficient and robust analytical methods. The sampling of the water from the site of pollution also plays a vital role in monitoring and determining the extent of pollution. The developed onsite sampling device shows the efficient usage of a novel idea of available materials to construct a well-structured way of sampling without much cost and labour.The μ-SPE methodology in the current study is quite sufficient to carry out fast extraction and subsequent analysis in the laboratory after sampling has been done on the site or area of study.

The comparison of the tedious Liquid-Liquid Extraction and the developed method showed they produced almost the same results, however the LLE is tedious and large volume of organic solvents are generated in this method.

The significant concentrations of especially Endrin reported in this study are potential threats to the local community and ecosystems in proximity including the aquatic life. These OCPs can be drained from areas of exposure into the nearby water bodies and reach the non-target organisms. The problem might be intensified with the lack of education and awareness about the toxic effect of pesticide exposure in the studied area. Additionally, the ability of these pesticides to undergo

long-range global transport poses an international concern about the residue detected at any corner of the globe.

The presence and detection of the target analyte OCPs in the sampled sea foods signifies the danger and potential threat of the pesticides to the human consumption and subsequent bioaccumulation in the body. The study on the sea foods also showed substantial presence of Endrin in the some sea foods with other target analytes also being detected.

5.2 RECOMMENDATIONS

Recommendation1: We recommend that change of the motor of the device to an electric one rather than battery operation. That way it will have more efficiency and less like hood of stoppage due to lack of power.

Recommendation 2: We also recommend continuously monitoring of both water and sea food for elaborate detection of any pollution threat.

Recommendation 3: As one of the purposes behind developing this system was to involve developing countries in the monitoring of their local pollution levels, affordability was taken into consideration and achieved by improvising from items used in daily lives. Therefore we recommend use of the developed system.

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